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(54) Title: MORPHOGENIC PROTEIN SOLUBLE COMPLEX AND COMPOSITION THEREOF (57) Abstract Disclosed are compositions of morphogenic proteins constituting soluble forms of these proteins, antibodies that distinguish between soluble and mature forms, and method for producing these morphogenic proteins and antibodies.		

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MORPHOGENIC PROTEIN SOLUBLE COMPLEX AND COMPOSITION THEREOF.Field of the Invention

The present invention relates generally to
5 morphogenic proteins and, more particularly, to
compositions having improved solubility in aqueous
solvents.

Background of the Invention

10 Morphogenic proteins ("morphogens") are well known
and described in the art. See, for example, U.S. Pat.
Nos. 4, 968,590; 5,011,691; 5,018,753; PCT US92/01968 and
PCT US92/07432; as well as various articles published in
the scientific literature, including Ozkaynak et al.
15 (1992) J.Biol. Chem. 267:25220-25227 and Ozkaynak et al.
(1991) Biochem. Biophys. Res. Comm. 179:116-123. The
art has described how to isolate morphogenic proteins
from bone, how to identify genes encoding these proteins
and how to express them using recombinant DNA technology.
20 The morphogenic proteins are capable of inducing
endochondral bone formation and other tissue formation in
a mammal when they are properly folded, dimerized and
disulfide bonded to produce a dimeric species having the
appropriate three dimensional conformation. The proteins
25 have utility in therapeutic applications, either by
direct or systemic administration. Where bone induction
is desired, for example, the morphogen typically is
provided to the desired site for bone formation in a
mammal in association with a suitable matrix having the
30 appropriate conformation to allow the infiltration,
proliferation and differentiation of migrating progenitor
cells. The morphogenic protein adsorbed to the surfaces

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of a suitable matrix is generally referred to in the art as an osteogenic device. The proteins can be isolated from bone or, preferably, the gene encoding the protein is produced recombinantly in a suitable host cell.

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The morphogen precursor polypeptide chains share a common structural motif, including a N-terminal signal sequence and pro region, both of which are cleaved to produce a mature sequence, capable of disulfide bonding and comprising an N-terminal extension and a C-terminal domain whose amino acid sequence is highly conserved among members of the family. In their mature dimeric forms, the morphogens typically are fairly insoluble under physiological conditions. Increasing the solubility of these proteins has significant medical utility as it would enhance systemic administration of morphogens as therapeutics. Various carrier proteins, including serum albumin and casein are known to increase the solubility of morphogens (see, for example, PCT US92/07432). PCT US92/05309 (WO 93/00050) discusses the use of various solubilizing agents, including various amino acids and methyl esters thereof, as well as guanidine, sodium chloride and heparin, to increase the solubility of mature dimeric BMP2.

25

Improved methods for the recombinant expression of morphogenic proteins is an ongoing effort in the art. It is an object of this invention to provide an improvement in the methods for producing and purifying morphogenic proteins having high specific activity, and for formulating compositions and osteogenic devices comprising these proteins. Another object is to provide soluble forms of morphogenic proteins consisting essentially of amino acid sequences derived from

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morphogenic proteins. Another object is to provide formulations which stabilize the soluble complex of morphogenic proteins. Still another object is to provide means for distinguishing between soluble forms of the protein and the mature morphogenic species, to provide means for quantitating the amounts of these proteins in a fluid, including a body fluid, such as serum, cerebro-sprinal fluid or peritoneal fluid, and to provide polyclonal and monoclonal antibodies capable of distinguishing between these various species.

Another object is to provide antibodies and biological diagnostic assays for monitoring the concentration of morphogens and endogenous anti-morphogen antibodies present in a body fluid and to provide kits and assays for detecting fluctuations in the concentrations of these proteins in a body fluid. U.S. Patent No. 4,857,456 and Urist et al. (1984) Proc. Soc. Exp. Biol. Med. 176:472-475 describe a serum assay for detecting a protein purported to be a bone morphogenetic protein. The protein is not a member of the morphogen family of proteins described herein, differing in molecular weight, structural characteristics and solubility from these proteins.

Summary of the Invention

It now has been discovered that morphogenic protein secreted into cultured medium from mammalian cells contains as a significant fraction of the secreted protein a soluble form of the protein, and that this soluble form comprises the mature dimeric species, including truncated forms thereof, noncovalently associated with at least one, and preferably two pro domains. It further has been discovered that antibodies

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can be used to discriminate between these two forms of the protein. These antibodies may be used as part of a purification scheme to selectively isolate the mature or the soluble form of morphogenic protein, as well as to
5 quantitate the amount of mature and soluble forms produced. These antibodies also may be used as part of diagnostic treatments to monitor the concentration of morphogenic proteins in solution in a body and to detect
10 fluctuations in the concentration of the proteins in their various forms. The antibodies and proteins also may be used in diagnostic assays to detect and monitor concentrations of endogenous anti-morphogen antibodies to the various forms of these proteins in the body.

15 An important embodiment of the invention is a dimeric protein comprising a pair of polypeptide subunits associated to define a dimeric structure having morphogenic activity. As defined herein and in parent, related applications, morphogens generally are capable
20 of all of the following biological functions in a morphogenically permissive environment: stimulating proliferation of progenitor cells; stimulating the differentiation of progenitor cells; stimulating the proliferation of differentiated cells; and supporting the
25 growth and maintenance of differentiated cells.

Each of the subunits of the dimeric morphogenic protein comprises at least the 100 amino acid peptide sequence having the pattern of seven or more cysteine
30 residues characteristic of the morphogen family. Preferably, at least one of the subunits comprises the mature form of a subunit of a member of the morphogen family, or an allelic, species, chimeric or other sequence variant thereof, noncovalently complexed with a

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peptide comprising part or all of a pro region of a member of the morphogen family, or an allelic, species, chimeric or other sequence variant thereof. The pair of subunits and one or, preferably, two pro region peptides, together form a complex which is more soluble in aqueous solvents than the uncomplexed pair of subunits.

Preferably, both subunits comprise a mature form of a subunit of a member of the morphogen family or an allelic, species, chimeric or other sequence variant thereof, and both subunits are noncovalently complexed with a peptide comprising a pro region, or a fragment thereof. Most preferably, each subunit is the mature form of human OP-1, or a species, allelic or other sequence variant thereof, and the pro region peptide is the entire or partial sequence of the pro region of human OP-1, or a species, allelic, chimeric or other sequence variant thereof. Currently, preferred pro regions are full length forms of the pro region. Pro region fragments preferably include the first 18 amino acids of the pro sequence. Other useful pro region fragments are truncated sequences of the intact pro region sequence, the truncation occurring at the proteolytic cleavage site Arg-Xaa-Xaa-Arg. As will be appreciated by those having ordinary skill in the art, useful sequences encoding the pro region may be obtained from genetic sequences encoding known morphogens. Alternatively, chimeric pro regions can be constructed from the sequences of one or more known morphogens. Still another option is to create a synthetic sequence variant of one or more known pro region sequences.

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As used herein, the mature form of a morphogen protein subunit includes the intact C-terminal domain and intact or truncated forms of the N-terminal extensions. For example, useful mature forms of OP-1 include dimeric species defined by residues 293-431 of Seq ID No. 1, as well as truncated sequences thereof, including sequences defined by residues 300-431, 313-431, 315-431, 316-431 and 318-431. Note that this last sequence retains only about the last 10 residues of the N-terminal extension sequence. Fig. 2 presents the N-terminal extensions for a number of preferred morphogen sequences. Canonical Arg-Xaa-Xaa-Arg cleavage sites where truncation may occur are boxed or underlined in the figure. As will be appreciated by those having ordinary skill in the art, mature dimeric species may include subunit combinations having different N-terminal truncations.

Other soluble forms of morphogens include dimers of the uncleaved pro forms of these proteins (see below), as well as "hemi-dimers" wherein one subunit of the dimer is an uncleaved pro form of the protein, and the other subunit comprises the mature form of the protein, including truncated forms thereof, preferably noncovalently associated with a cleaved pro domain.

The soluble proteins of this invention also are useful in the formation of therapeutic compositions for administration to a mammal, particularly a human, and for the development of biological assays for monitoring the concentration of these proteins and endogenous antibodies to these proteins in cell samples and body fluids, including, but not limited to, serum, cerebrospinal fluid and peritoneal fluid.

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The foregoing and other objects, features and advantages of the present invention will be made more apparent from the following detailed description of the invention.

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Brief Description of the Drawings

Fig. 1 is a schematic representation of a morphogen polypeptide chain as expressed from a nucleic acid
10 encoding the sequence, wherein the cross-hatched region represents the signal sequence; the stippled region represents the pro domain; the hatched region represents the N-terminus ("N-terminal extension") of the mature protein sequence; and the open region represents the
15 C-terminal region of the mature protein sequence defining the conserved seven cysteine domain, the conserved cysteines being indicated by vertical hatched lines;

Fig.2 lists the sequences of the N-terminal
20 extensions of the mature forms of various morphogens; and

Fig. 3 is a gel filtration column elution profile of a soluble morphogen (OP-1) produced and purified from a mammalian cell culture by IMAC, S-Sepharose and S-200HR
25 chromatography in TBS (Tris-buffered saline), wherein V_0 is the void volume, ADH is alcohol dehydrogenase (MW 150 kDa), BSA is bovine serum albumin (MW 67 kDa), CA is carbonic anhydrase (MW 29kDa) and CytC is cytochrome C (MW 12.5 kDa).

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Detailed Description

A soluble form of morphogenic proteins now has been discovered wherein the proteins consist essentially of the amino acid sequence of the protein. The soluble form is a non-covalently associated complex comprising the pro domain or a fragment thereof, noncovalently associated or complexed with a dimeric protein species having morphogenic activity, each polypeptide of the dimer having less than 200 amino acids and comprising at least the C-terminal six, and preferably seven cysteine skeleton defined by residues 330-431 and 335-431, respectively, of Seq. ID No. 1. Preferably, the polypeptide chains of the dimeric species comprise the mature forms of these sequences, or truncated forms thereof. Preferred truncated forms comprise the intact C-terminal domain and at least 10 amino acids of the N-terminal extension sequence. The soluble forms of these morphogenic proteins may be isolated from cultured cell medium, a mammalian body fluid, or may be formulated in vitro.

In vivo, under physiological conditions, the pro domain may serve to enhance the transportability of the proteins, and/or to protect the proteins from proteases and scavenger molecules, including antibodies. The pro domains also may aid in targeting the proteins to a particular tissue and/or to present the morphogen to a morphogen cell surface receptor by interaction with a co-receptor molecule. The isolated proteins may be used

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in therapeutic formulations, particularly for oral or parenteral administration, and in the development of diagnostic and other tissue evaluating kits and assays to monitor the level of endogenous morphogens and endogenous anti-morphogen antibodies.

Detailed descriptions of the utility of these morphogens in therapies to regenerate lost or damaged tissues and/or to inhibit the tissue destructive effects of tissue disorders or diseases, are provided in international applications US92/01968 (WO92/15323); US92/07358 (WO93/04692) and US92/07432 (WO93/05751) the disclosures of which are incorporated herein by reference. Morphogens, including the soluble morphogen complexes of this invention, are envisioned to have particular utility as part of therapies for regenerating lost or damaged bone, dentin, periodontal, liver, cardiac, lung and nerve tissue, as well as for protecting these tissues from the tissue destructive effects associated with an immunological response. The proteins also are anticipated to provide a tissue protective effect in the treatment of metabolic bone disorders, such as osteoporosis, osteomalacia and osteosarcoma; in the treatment of liver disorders, including cirrhosis, hepatitis, alcohol liver disease and hepatic encephalopathy; and in the treatment or prevention of ischemia reperfusion-associated tissue damage, particularly to nerve or cardiac tissue.

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Presented below are detailed descriptions of useful soluble morphogen complexes of this invention, as well as how to make and use them.

5 I. Useful Soluble Morphogen Complexes - Protein Considerations

Among the morphogens useful in this invention are proteins originally identified as osteogenic proteins,
10 such as the OP-1, OP-2 and CBMP2 proteins, as well as amino acid sequence-related proteins such as DPP (from Drosophila), Vgl (from Xenopus), Vgr-1 (from mouse, see U.S. 5,011,691 to Oppermann et al.), GDF-1 (from mouse, see Lee (1991) PNAS 88:4250-4254), 60A protein (from
15 Drosophila, Seq. ID No. 24, see Wharton et al. (1991) PNAS 88:9214-9218), and the recently identified OP-3.

The members of this family, which are a subclass of the TGF- β super-family of proteins, share characteristic
20 structural features, represented schematically in Fig. 1, as well as substantial amino acid sequence homology in their C-terminal domains, including a conserved seven cysteine structure. As illustrated in the figure, the proteins are translated as a precursor polypeptide
25 sequence 10, having an N-terminal signal peptide sequence 12, (the "pre pro" region, indicated in the figure by cross-hatching), typically less than about 30 residues, followed by a "pro" region 14, indicated in the figure by stippling, and which is cleaved to yield the mature
30 sequence 16. The mature sequence comprises both the conserved C-terminal seven cysteine domain 20, and an N-terminal sequence 18, referred to herein as an N-terminal extension, and which varies significantly in sequence between the various morphogens. Cysteines are

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represented in the figure by vertical hatched lines 22.
The polypeptide chains dimerize and these dimers
typically are stabilized by at least one interchain
disulfide bond linking the two polypeptide chain
5 subunits.

The signal peptide is cleaved rapidly upon
translation, at a cleavage site that can be predicted in
a given sequence using the method of Von Heijne ((1986)
10 Nucleic Acids Research 14:4683-4691.) The "pro" form of
the protein subunit, 24, in Fig. 1, includes both the pro
domain and the mature domain, peptide bonded together.
Typically, this pro form is cleaved while the protein is
still within the cell, and the pro domain remains
15 noncovalently associated with the mature form of the
subunit to form a soluble species that appears to be the
primary form secreted from cultured mammalian cells.
Typically, previous purification techniques utilized
denaturing conditions that disassociated the complex.

20 Other soluble forms of morphogens secreted from
mammalian cells include dimers of the pro forms of these
proteins, wherein the pro region is not cleaved from the
mature domain, and "hemi-dimers", wherein one subunit
25 comprises a pro form of the polypeptide chain subunit and
the other subunit comprises the cleaved mature form of
the polypeptide chain subunit (including truncated forms
thereof), preferably noncovalently associated with a
cleaved pro domain.

30 The isolated pro domain typically has a substantial
hydrophobic character, as determined both by analysis of
the sequence and by characterization of its properties in
solution. The isolated pro regions alone typically are

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not significantly soluble in aqueous solutions, and require the presence of denaturants, e.g., detergents, urea, guanidine HCl, and the like, and/or one or more carrier proteins. Accordingly, without being limited to
5 any given theory, the non-covalent association of the cleaved pro region with the mature morphogen dimeric species likely involves interaction of a hydrophobic portion of the pro region with a corresponding hydrophobic region on the dimeric species, the
10 interaction of which effectively protects or "hides" an otherwise exposed hydrophobic region of the mature dimer from exposure to aqueous environments, enhancing the affinity of the mature dimer species for aqueous solutions.

15

Morphogens comprise a subfamily of proteins within the TGF- β superfamily of structurally related proteins. Like the morphogens described herein, TGF- β also has a pro region which associates non-covalently with the
20 mature TGF- β protein form. However, unlike the morphogens, the TGF- β pro region contains numerous cysteines and forms disulfide bonds with a specific binding protein. The TGF- β 1 pro domain also is phosphorylated at one or more mannose residues, while the
25 morphogen pro regions typically are not.

Useful pro domains include the full length pro regions described below, as well as various truncated forms hereof, particularly truncated forms cleaved at
30 proteolytic Arg-Xaa-Xaa-Arg cleavage sites. For example, in OP-1, possible pro sequences include sequences defined by residues 30-292 (full length form); 48-292; and 158-292. Soluble OP-1 complex stability is enhanced when the pro region comprises the full length form rather than

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a truncated form, such as the 48-292 truncated form, in that residues 30-47 show sequence homology to the N-terminal portions of other morphogens, and are believed to have particular utility in enhancing complex stability for all morphogens. Accordingly, currently preferred pro sequences are those encoding the full length form of the pro region for a given morphogen (see below). Other pro sequences contemplated to have utility include biosynthetic pro sequences, particularly those that incorporate a sequence derived from the N-terminal portion of one or more morphogen pro sequences.

Table I, below, describes the various preferred morphogens identified to date, including their nomenclature as used herein, the sequences defining the various regions of the subunit sequences, their Seq. ID references, and publication sources for their nucleic acid and amino acid sequences. The disclosure of these publications is incorporated herein by reference. The mature protein sequences defined are the longest anticipated forms of these sequences. As described above, shorter, truncated forms of these sequences also are contemplated. Preferably, truncated mature sequences include at least 10 amino acids of the N-terminal extension. Fig. 2 lists the N-terminal extensions for a number of the preferred morphogen sequences described below. Arg-Xaa-Xaa-Arg cleavage sites that may yield truncated sequences of the mature subunit form are boxed or underlined in the figure.

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TABLE I

	"OP-1"	<p data-bbox="592 491 1396 1438">Refers generically to the group of morphogenically active proteins expressed from part or all of a DNA sequence encoding OP-1 protein, including allelic and species variants thereof, e.g., human OP-1 ("hOP-1"), or mouse OP-1 ("mOP-1"). The cDNA sequences and the amino acids encoding the full length proteins are provided in Seq. Id Nos. 1 and 2 (hOP1) and Seq. ID Nos. 3 and 4 (mOP1.) The mature proteins are defined by residues 293-431 (hOP1) and 292-430 (mOP1), wherein the conserved seven cysteine skeleton is defined by residues 330-431 and 329-430, respectively, and the N-terminal extensions are defined by residues 293-329 and 292-329, respectively. The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins, are defined essentially by residues 30-292 (hOP1) and residues 30-291 (mOP1).</p>
25	"OP-2"	<p data-bbox="592 1491 1396 1866">refers generically to the group of active proteins expressed from part or all of a DNA sequence encoding OP-2 protein, including allelic and species variants thereof, e.g., human OP-2 ("hOP-2") or mouse OP-2 ("mOP-2"). The full length proteins are provided in Seq. ID Nos. 5 and 6 (hOP2) and Seq. ID Nos. 7 and 8 (mOP2.) The mature proteins are defined</p>

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essentially by residues 264-402 (hOP2) and
261-399 (mOP2), wherein the conserved
seven cysteine skeleton is defined by
residues 301-402 and 298-399,
5 respectively, and the N-terminal
extensions are defined by residues 264-300
and 261-297, respectively. The "pro"
regions of the proteins, cleaved to yield
the mature, morphogenically active
10 proteins likely are defined essentially by
residues 18-263 (hOP2) and residues 18-260
(mOP2). (Another cleavage site also
occurs 21 residues upstream for both OP-2
proteins.)
15
"OP-3" refers generically to the group of active
proteins expressed from part or all of a
DNA sequence encoding OP-3 protein,
including allelic and species variants
20 thereof, e.g., mouse OP-3 ("mOP-3".) The
full length protein is provided in Seq. ID
No. 9. The mature protein is defined
essentially by residues 261-399 or
264-399, wherein the conserved seven
25 cysteine skeleton is defined by residues
298-399 and the N-terminal extension is
defined by residues 264-297 or 261-297.
The "pro" region of the protein, cleaved
to yield the mature, morphogenically
30 active proteins likely is defined
essentially by residues 20-262.

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"BMP2/BMP4" refers to protein sequences encoded by the human BMP2 and BMP4 genes. The amino acid sequence for the full length proteins, referred to in the literature as BMP2A and BMP2B, or BMP2 and BMP4, appear in Seq. ID Nos. 10 and 11, respectively, and in Wozney, et al. (1988) Science 242:1528-1534. The pro domain for BMP2 (BMP2A) likely includes residues 25-248 or 25-282; the mature protein, residues 249-396 or 283-396, of which residues 249-296/283-296 define the N-terminal extension and 295-396 define the C-terminal domain. The pro domain for BMP4 (BMP2B) likely includes residues 25-256 or 25-292; the mature protein, residues 257-408 or 293-408, of which 257-307/293-307 define the N-terminal extension, and 308-408 define the C-terminal domain.

"DPP" refers to protein sequences encoded by the Drosophila DPP gene. The amino acid sequence for the full length protein, including the mature form and the pro region, appears in Seq.ID No. 12 and in Padgett, et al (1987) Nature 325: 81-84. The pro domain likely extends from the signal peptide cleavage site to residue 456; the mature protein likely is defined by residues 457-588, where residues 457-586 define the N-terminal extension and 487-588 define the C-terminal domain.

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- "Vgl" refers to protein sequences encoded by the Xenopus Vgl gene. The amino acid sequence for the full length protein, including the mature form and the pro region, appears in Seq.ID No. 13 and in Weeks (1987) Cell 51: 861-867. The pro domain likely extends from the signal peptide cleavage site to residue 246; the mature protein likely is defined by residues 247-360, where residues 247-258 define the N-terminal extension, and residues 259-360 define the C-terminal domain.
- "Vgr-1" refers to protein sequences encoded by the murine Vgr-1 gene. The amino acid sequence for the full length protein, including the mature form and the pro region, appears in Seq. ID No. 14 and in Lyons, et al, (1989) PNAS 86: 4554-4558. The pro domain likely extends from the signal peptide cleavage site to residue 299; the mature protein likely is defined by residues 300-438, where residues 300-336 define the N-terminal extension and residues 337-438 define the C-terminus.
- "GDF-1" refers to protein sequences encoded by the human GDF-1 gene. The cDNA and encoded amino sequence for the full length protein is provided in Seq. ID. No. 15 and Lee (1991) PNAS 88:4250-4254. The pro domain

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likely extends from the signal peptide cleavage site to residue 214; the mature protein likely is defined by residues 215-372, where residues 215-256 define the N-terminal extension and residues 257-372 define the C-terminus.

"60A" refers to protein sequences encoded by the *Drosophila* 60A gene. The amino acid sequence for the full length protein appears in Seq. ID No. 16 and in Wharton et al. (1991) PNAS 88:9214-9218) The pro domain likely extends from the signal peptide cleavage site to residue 324; the mature protein likely is defined by residues 325-455, wherein residues 325-353 define the N-terminal extension and residues 354-455 define the C-terminus.

"BMP3" refers to protein sequences encoded by the human BMP3 gene. The amino acid sequence for the full length protein, including the mature form and the pro region, appears in Seq.ID No. 17 and in Wozney et al. (1988) Science 242: 1528-1534. The pro domain likely extends from the signal peptide cleavage site to residue 290; the mature protein likely is defined by residues 291-472, wherein residues 291-370 define the N-terminal extension and residues 371-472 define the C-terminus.

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- "BMP5" refers to protein sequences encoded by the human BMP5 gene. The amino acid sequence for the full length protein, including the mature form and the pro region, appears in Seq.ID No. 18 and in Celeste, et al. (1990) PNAS 87: 9843-9847. The pro domain likely extends from the signal peptide cleavage site to residue 316; the mature protein likely is defined by residues 317-454, where residues 317-352 define the N-terminus and residues 352-454 define the C-terminus.
- "BMP6" refers to protein sequences encoded by the human BMP6 gene. The amino acid sequence for the full length protein, including the mature form and the pro region, appears in Seq. ID No. 16 and in Celeste, et al. (1990) PNAS 87: 9843-5847. The pro domain likely includes extends from the signal peptide cleavage site to residue 374; the mature sequence likely includes residues 375-513, where residues 375-411 define the N-terminus and residues 412-513 define the C-terminus.

Note that the OP-2 and OP-3 proteins have an additional cysteine residue in the C-terminal region (e.g., see residue 338 in these sequences), in addition to the conserved cysteine skeleton in common with the other proteins in this family. The GDF-1 protein has a four amino acid insert within the conserved skeleton

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("Gly-Gly-Pro-Pro") but this insert likely does not interfere with the relationship of the cysteines in the folded structure. In addition, the CBMP2 proteins are missing one amino acid residue within the cysteine
5 skeleton.

The dimeric morphogen species are inactive when reduced, but are active as oxidized homodimers and when oxidized in combination with other morphogens of this
10 invention. Thus, as defined herein, a morphogen useful in a soluble morphogen complex is a dimeric protein comprising a pair of polypeptide chains, wherein each polypeptide chain has less than 200 amino acids and comprises at least the C-terminal six, preferably seven
15 cysteine skeleton defined by residues 335-431 of Seq. ID No. 1, including functionally equivalent arrangements of these cysteines (e.g., amino acid insertions or deletions which alter the linear arrangement of the cysteines in the sequence but not
20 their relationship in the folded structure), such that, when the polypeptide chains are folded, the dimeric protein species comprising the pair of polypeptide chains has the appropriate three-dimensional structure, including the appropriate intra- or inter-chain
25 disulfide bonds such that the protein is capable of acting as a morphogen as defined herein. The solubility of these structures is improved when the mature dimeric form of a morphogen, in accordance with the invention, is complexed with at least one, and
30 preferably two, pro domains.

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Various generic sequences (Generic Sequence 1-6) defining preferred C-terminal sequences useful in the soluble morphogens of this invention are described in USSN 07/923,780, incorporated herein above by
5 reference. Two currently preferred generic sequences are described below.

Generic Sequence 7 (Seq. ID No. 20) and Generic Sequence 8 (Seq. ID No. 21) disclosed below,
10 accommodate the homologies shared among preferred morphogen protein family members identified to date, including OP-1, OP-2, OP-3, CBMP2A, CBMP2B, BMP3, 60A, DPP, Vg1, BMP5, BMP6, Vrg-1, and GDF-1. The amino acid sequences for these proteins are described herein (see
15 Sequence Listing) and/or in the art, as well as in PCT publication US 92/07358, (WO93/04692), for example. The generic sequences include both the amino acid identity shared by these sequences in the C-terminal domain, defined by the six and seven cysteine skeletons
20 (Generic Sequences 7 and 8, respectively), as well as alternative residues for the variable positions within the sequence. The generic sequences allow for an additional cysteine at position 41 (Generic Sequence 7) or position 46 (Generic Sequence 8), providing an
25 appropriate cysteine skeleton where inter- or intramolecular disulfide bonds can form, and containing certain critical amino acids which influence the tertiary structure of the proteins.

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Generic Sequence 7

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          Leu Xaa Xaa Xaa Phe
              1               5
5      Xaa Xaa Xaa Gly Trp Xaa Xaa Xaa Xaa
              10
      Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala
          15               20
      Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
10              25               30
      Xaa Pro Xaa Xaa Xaa Xaa Xaa
              35
      Xaa Xaa Xaa Asn His Ala Xaa Xaa
          40               45
15      Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
              50
      Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
          55               60
      Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
20              65
      Xaa Xaa Xaa Leu Xaa Xaa Xaa
          70               75
      Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
              80
25      Xaa Xaa Xaa Xaa Met Xaa Val Xaa
          85               90
      Xaa Cys Xaa Cys Xaa
              95

```

wherein each Xaa is independently selected from a group
 30 of one or more specified amino acids defined as
 follows: "Res." means "residue" and Xaa at res.2 =
 (Tyr or Lys); Xaa at res.3 = Val or Ile); Xaa at res.4
 = (Ser, Asp or Glu); Xaa at res.6 = (Arg, Gln, Ser, Lys
 or Ala); Xaa at res.7 = (Asp or Glu); Xaa at res.8 =

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(Leu, Val or Ile); Xaa at res.11 = (Gln, Leu, Asp, His, Asn or Ser); Xaa at res.12 = (Asp, Arg, Asn or Glu); Xaa at res. 13 = (Trp or Ser); Xaa at res.14 = (Ile or Val); Xaa at res.15 = (Ile or Val); Xaa at res.16 (Ala or Ser); Xaa at res.18 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.19 = (Gly or Ser); Xaa at res.20 = (Tyr or Phe); Xaa at res.21 = (Ala, Ser, Asp, Met, His, Gln, Leu or Gly); Xaa at res.23 = (Tyr, Asn or Phe); Xaa at res.26 = (Glu, His, Tyr, Asp, Gln, Ala or Ser); Xaa at res.28 = (Glu, Lys, Asp, Gln or Ala); Xaa at res.30 = (Ala, Ser, Pro, Gln, Ile or Asn); Xaa at res.31 = (Phe, Leu or Tyr); Xaa at res.33 = (Leu, Val or Met); Xaa at res.34 = (Asn, Asp, Ala, Thr or Pro); Xaa at res.35 = (Ser, Asp, Glu, Leu, Ala or Lys); Xaa at res.36 = (Tyr, Cys, His, Ser or Ile); Xaa at res.37 = (Met, Phe, Gly or Leu); Xaa at res.38 = (Asn, Ser or Lys); Xaa at res.39 = (Ala, Ser, Gly or Pro); Xaa at res.40 = (Thr, Leu or Ser); Xaa at res.44 = (Ile, Val or Thr); Xaa at res.45 = (Val, Leu, Met or Ile); Xaa at res.46 = (Gln or Arg); Xaa at res.47 = (Thr, Ala or Ser); Xaa at res.48 = (Leu or Ile); Xaa at res.49 = (Val or Met); Xaa at res.50 = (His, Asn or Arg); Xaa at res.51 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.52 = (Ile, Met, Asn, Ala, Val, Gly or Leu); Xaa at res.53 = (Asn, Lys, Ala, Glu, Gly or Phe); Xaa at res.54 = (Pro, Ser or Val); Xaa at res.55 = (Glu, Asp, Asn, Gly, Val, Pro or Lys); Xaa at res.56 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser, Gly, Ile or His); Xaa at res.57 = (Val, Ala or Ile); Xaa at res.58 = (Pro or Asp); Xaa at res.59 = (Lys, Leu or Glu); Xaa at res.60 = (Pro, Val or Ala); Xaa at res.63 = (Ala or Val); Xaa at res.65 = (Thr, Ala or Glu); Xaa at res.66 = (Gln, Lys, Arg or Glu); Xaa at res.67 = (Leu, Met or Val); Xaa at res.68 = (Asn, Ser, Asp or Gly); Xaa at

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res.69 = (Ala, Pro or Ser); Xaa at res.70 = (Ile, Thr, Val or Leu); Xaa at res.71 = (Ser, Ala or Pro); Xaa at res.72 = (Val, Leu, Met or Ile); Xaa at res.74 = (Tyr or Phe); Xaa at res.75 = (Phe, Tyr, Leu or His); Xaa at
5 res.76 = (Asp, Asn or Leu); Xaa at res.77 = (Asp, Glu, Asn, Arg or Ser); Xaa at res.78 = (Ser, Gln, Asn, Tyr or Asp); Xaa at res.79 = (Ser, Asn, Asp, Glu or Lys); Xaa at res.80 = (Asn, Thr or Lys); Xaa at res.82 = (Ile, Val or Asn); Xaa at res.84 = (Lys or Arg); Xaa at
10 res.85 = (Lys, Asn, Gln, His, Arg or Val); Xaa at res.86 = (Tyr, Glu or His); Xaa at res.87 = (Arg, Gln, Glu or Pro); Xaa at res.88 = (Asn, Glu, Trp or Asp); Xaa at res.90 = (Val, Thr, Ala or Ile); Xaa at res.92 = (Arg, Lys, Val, Asp, Gln or Glu); Xaa at res.93 = (Ala, Gly, Glu or Ser); Xaa at res.95 = (Gly or Ala) and Xaa
15 at res.97 = (His or Arg).

As described above, Generic Sequence 8 (Seq. ID No. 21) includes all of Generic Sequence 7 and in addition includes the following sequence at its N-terminus:

Cys Xaa Xaa Xaa Xaa
1 5

25 Accordingly, beginning with residue 7, each "Xaa"
in Generic Seq. 8 is a specified amino acid defined as
for Generic Seq. 7, with the distinction that each
residue number described for Generic Sequence 7 is
shifted by five in Generic Seq. 8. Thus, "Xaa at res.2
30 =(Tyr or Lys)" in Gen. Seq. 7 refers to Xaa at res. 7

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in Generic Seq. 8. In Generic Seq. 8, Xaa at res.2 = (Lys, Arg, Ala or Gln); Xaa at res.3 = (Lys, Arg or Met); Xaa at res.4 = (His, Arg or Gln); and Xaa at res.5 = (Glu, Ser, His, Gly, Arg, Pro, Thr, or Tyr).

5
Accordingly, other useful sequences defining preferred C-terminal sequences are those sharing at least 70% amino acid sequence homology or "similarity", and preferably 80% homology or similarity with any of
10 the sequences incorporated into Generic Seq. 7 and 8 above. These are anticipated to include allelic, species, chimeric and other sequence variants, (e.g., including "muteins" or "mutant proteins"), whether naturally-occurring or biosynthetically produced, as
15 well as novel members of this morphogenic family of proteins. As used herein, "amino acid sequence homology" is understood to mean amino acid sequence similarity, and homologous sequences share identical or similar amino acids, where similar amino acids are
20 conserved amino acids as defined by Dayoff et al., Atlas of Protein Sequence and Structure; vol.5, Suppl.3, pp.345-362 (M.O. Dayoff, ed., Nat'l BioMed. Research Fdn., Washington D.C. 1978.) Thus, a candidate sequence sharing 70% amino acid homology with
25 a reference sequence requires that, following alignment of the candidate sequence with the reference sequence, 70% of the amino acids in the candidate sequence are identical to the corresponding amino acid in the reference sequence, or constitute a conserved amino
30 acid change thereto. "Amino acid sequence identity" is understood to require identical amino acids between two

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aligned sequences. Thus, a candidate sequence sharing 60% amino acid identity with a reference sequence requires that, following alignment of the candidate sequence with the reference sequence, 60% of the amino acids in the candidate sequence are identical to the corresponding amino acid in the reference sequence.

As used herein, all homologies and identities calculated use OP-1 as the reference sequence. Also as used herein, sequences are aligned for homology and identity calculations using the method of Needleman et al. (1970) J.Mol. Biol. 48:443-453 and identities calculated by the Align program (DNASTAR, Inc.) In all cases, internal gaps and amino acid insertions in the candidate sequence as aligned are ignored when making the homology/identity calculation.

Also as used herein, "sequence variant" is understood to mean an amino acid sequence variant form of the morphogen protein, wherein the amino acid change or changes in the sequence do not alter significantly the morphogenic activity (e.g., tissue regeneration activity) of the protein, and the variant molecule performs substantially the same function in substantially the same way as the naturally-occurring form of the molecule. Sequence variants may include single or multiple amino acid changes, and are intended to include chimeric sequences as described below. The variants may be naturally-occurring or may be biosynthetically induced by using standard recombinant DNA techniques or chemical protein synthesis methodologies.

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The currently most preferred protein sequences useful in soluble morphogen complexes in this invention include those having greater than 60% identity, preferably greater than 65% identity, with the amino acid sequence defining the conserved six cysteine skeleton of hOP1 (e.g., residues 335-431 of Seq. ID No. 5). These most preferred sequences include both allelic and species variants of the OP-1 and OP-2 proteins, including the *Drosophila* 60A protein.

Accordingly, in another preferred aspect of the invention, useful morphogens include active proteins comprising species of polypeptide chains having the generic amino acid sequence herein referred to as "OPX", which accommodates the homologies between the various identified species of OP1 and OP2 (Seq. ID No. 22).

In still another preferred aspect of the invention, useful morphogens include active proteins comprising amino acid sequences encoded by nucleic acids that hybridize to DNA or RNA sequences encoding the conserved C-terminal cysteine domain of OP1 or OP2, e.g., defined by nucleotides 1036-1341 and nucleotides 1390-1695 of Seq. ID Nos. 1 and 5, respectively, under stringent hybridization conditions. As used herein, stringent hybridization conditions are defined as hybridization in 40% formamide, 5 X SSPE, 5 X Denhardt's Solution, and 0.1% SDS at 37°C overnight, and washing in 0.1 X SSPE, 0.1% SDS at 50°C.

Similarly, in another preferred aspect of the invention, useful pro region peptides include polypeptide chains comprising amino acid sequences encoded by nucleic acids that hybridize to DNA or RNA sequences encoding at least the N-terminal 18 amino

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acids of the pro region sequences for any of the sequences listed in Seq. ID Nos. 1-19, under stringent hybridization conditions. Most preferably, the peptides are encoded by nucleic acids that hybridize to the DNA or RNA sequences encoding at least the N-terminal 18 amino acids of the pro region sequences for OP1 or OP2, e.g., nucleotides 136-192 and nucleotides 152-211 of Seq. ID Nos. 1 and 5, respectively.

10

Useful N-terminal extension sequences are listed in Fig. 2 for use with the C-terminal domains described above. Also as described above, the full length N-terminal extensions, or truncated forms thereof, may be used in preferred dimeric species. The mature dimeric species may be produced from intact DNAs, or truncated forms thereof. It also is envisioned as an embodiment of the invention that chimeric morphogen sequences can be used. Thus, DNAs encoding chimeric morphogens may be constructed using part or all of the N-terminal extension from one morphogen and a C-terminal domain derived from one or more other morphogens. These chimeric proteins may be synthesized using standard recombinant DNA methodology and/or automated chemical nucleic acid synthesis methodology well described in the art. Other chimeric morphogens include soluble morphogen complexes where the pro domain is encoded from a DNA sequence corresponding to one or more morphogen pro sequences, and part or all of the mature domain is encoded by DNA derived from one or more

30

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other, different morphogens. These soluble chimerics may be produced from a single synthetic DNA as described below, or, alternatively, may be formulated in vitro from isolated components also as described
5 herein below.

Finally, the morphogen pro domains and/or mature form N-terminal extensions themselves may be useful as tissue targeting sequences. As described above, the
10 morphogen family members share significant sequence homology in their C-terminal active domains. By contrast, the sequences diverge significantly in the sequences which define the pro domain and the N-terminal 39 amino acids of the mature protein.
15 Accordingly, the pro domain and/or N-terminal extension sequence may be morphogen-specific. Accordingly, part or all of these morphogen-specific sequences may serve as tissue targeting sequences for the morphogens described herein. For example, the N-terminal
20 extension and/or pro domains may interact specifically with one or more molecules at the target tissue to direct the morphogen associated with the pro domain to that tissue. Thus, for example, the morphogen-specific sequences of OP-1, BMP2 or BMP4, all of which proteins
25 are found naturally associated with bone tissue (see, for example, US Pat. No. 5,011,691) may be particularly useful sequences when the morphogen complex is to be targeted to bone. Similarly, BMP6 (or Vgr-1) specific sequences may be used when targeting to lung tissue is
30 desired. Alternatively, the morphogen-specific sequences of GDF-1 may be used to target soluble

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morphogen complexes to nerve tissue, particularly brain tissue, where GDF-1 appears to be primarily expressed (see, for example, Lee, PNAS, 88:4250-4254 (1991), incorporated herein by reference).

5

II. Recombinant Production of Soluble Morphogen Complexes

Soluble morphogen complexes can be produced from
10 eukaryotic host cells, preferably mammalian cells,
using standard recombinant expression techniques. An
exemplary protocol currently preferred, is provided
below, using a particular vector construct and chinese
hamster ovary (CHO) cell line. Those skilled in the
15 art will appreciate that other expression systems are
contemplated to be useful, including other vectors and
other cell systems, and the invention is not intended
to be limited to soluble morphogenic protein complexes
produced only by the method detailed hereinbelow.
20 Similar results to those described herein have been
observed using recombinant expression systems developed
for COS and BSC cells.

Morphogen DNA encoding the precursor sequence is
25 subcloned into an insertion site of a suitable,
commercially available pUC-type vector (e.g., pUC-19,
ATCC #37254, Rockville, MD), along with a suitable
promoter/enhancer sequences and 3' termination
sequences. Useful DNA sequences include the published
30 sequences encoding these proteins, and/or synthetic
constructs. Currently preferred promoter/enhancer
sequences are the CMV promoter (human cytomegalovirus
major intermediate - early promoter) and the mouse

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- mammary tumor virus promoter (mMTV) boosted by the rous sarcoma virus LTR enhancer sequence (e.g., from Clontech, Inc., Palo Alto). Expression also may be further enhanced using transactivating enhancer sequences. The plasmid also contains DHFR as an amplifiable marker, under SV40 early promoter control (ATCC #37148). Transfection, cell culturing, gene amplification and protein expression conditions are standard conditions, well known in the art, such as are described, for example in Ausubel et al., ed., Current Protocols in Molecular Biology, John Wiley & Sons, NY (1989). Briefly, transfected cells are cultured in medium containing 0.1-0.5% dialyzed fetal calf serum (FCS) and stably transfected high expression cell lines are obtained by subcloning and evaluated by standard Western or Northern blot. Southern blots also are used to assess the state of integrated sequences and the extent of their copy number amplification.
- 20 A currently preferred expression vector contains the DHFR gene, under SV40 early promoter control, as both a selection marker and as an inducible gene amplifier. The DNA sequence for DHFR is well characterized in the art, and is available commercially. For example, a suitable vector may be generated from pMAM-neo (Clontech, Inc., Palo Alto, CA) by replacing the neo gene (BamHI digest) with an SphI-BamHI, or a PvuII-BamHI fragment from pSV5-DHFR (ATCC #37148), which contains the DHFR gene under SV40 early promoter control. A BamHI site can be engineered at the SphI or PvuII site using standard techniques (e.g., by linker insertion or site-directed mutagenesis) to allow insertion of the fragment into the vector backbone. The morphogen DNA can be inserted into the

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polylinker site downstream of the MMTV-LTR sequence (mouse mammary tumor virus LTR). The CMV promoter sequence then may be inserted into the expression vector (e.g., from pCDM8, Invitrogen, Inc.) The SV40
5 early promoter, which drives DHFR expression, preferably is modified in these vectors to reduce the level of DHFR mRNA produced.

The currently preferred mammalian cell line is a
10 CHO Chinese hamster ovary, cell line, and the preferred procedure for establishing a stable morphogen production cell line with high expression levels comprises transfecting a stable CHO cell line, preferably CHO-DXB11, with the expression vector
15 described above, isolating clones with high morphogen expression levels, and subjecting these clones to cycles of subcloning using a limited dilution method described below to obtain a population of high expression clones. Subcloning preferably is performed
20 in the absence of MTX to identify stable high expression clones which do not require addition of MTX to the growth media for morphogen production.

In the subcloning protocol cells are seeded on ten
25 100mm petri dishes at a cell density of either 50 or 100 cells per plate, with or preferably without MTX in the culture media. After 14 days of growth, clones are isolated using cloning cylinders and standard procedures, and cultured in 24-well plates. Clones
30 then are screened for morphogen expression by Western

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immunoblots using standard procedures, and morphogen expression levels compared to parental lines. Cell line stability of high expression subclones then is determined by monitoring morphogen expression levels
5 over multiple cell passages (e.g., four or five passages).

III. Isolation of Soluble morphogen complex from conditioned media or body fluid

10

Morphogens are expressed from mammalian cells as soluble complexes. Typically, however the complex is disassociated during purification, generally by exposure to denaturants often added to the purification
15 solutions, such as detergents, alcohols, organic solvents, chaotropic agents and compounds added to reduce the pH of the solution. Provided below is a currently preferred protocol for purifying the soluble proteins from conditioned media (or, optionally, a body
20 fluid such as serum, cerebro-spinal or peritoneal fluid), under non-denaturing conditions. The method is rapid, reproducible and yields isolated soluble morphogen complexes in substantially pure form.

25 Soluble morphogen complexes can be isolated from conditioned media using a simple, three step chromatographic protocol performed in the absence of denaturants. The protocol involves running the media (or body fluid) over an affinity column, followed by
30 ion exchange and gel filtration chromatographies. The affinity column described below is a Zn-IMAC column. The present protocol has general applicability to the purification of a variety of morphogens, all of which are anticipated to be isolatable using only minor

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modifications of the protocol described below. An alternative protocol also envisioned to have utility an immunoaffinity column, created using standard procedures and, for example, using antibody specific
5 for a given morphogen pro domain (complexed, for example, to a protein A-conjugated Sepharose column.) Protocols for developing immunoaffinity columns are well described in the art, (see, for example, Guide to Protein Purification, M. Deutscher, ed., Academic
10 Press, San Diego, 1990, particularly sections VII and XI.)

In this experiment OP-1 was expressed in CHO cells as described above. The CHO cell conditioned media
15 containing 0.5% FBS was initially purified using Immobilized Metal-Ion Affinity Chromatography (IMAC). The soluble OP-1 complex from conditioned media binds very selectively to the Zn-IMAC resin and a high concentration of imidazole (50 mM imidazole, pH 8.0) is
20 required for the effective elution of the bound complex. The Zn-IMAC step separates the soluble OP-1 from the bulk of the contaminating serum proteins that elute in the flow through and 35 mM imidazole wash fractions. The Zn-IMAC purified soluble OP-1 is next
25 applied to an S-Sepharose cation-exchange column equilibrated in 20 mM NaPO_4 (pH 7.0) with 50 mM NaCl. This S-Sepharose step serves to further purify and concentrate the soluble OP-1 complex in preparation for the following gel filtration step. The protein was

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applied to a Sephacryl S-200HR column equilibrated in TBS. Using substantially the same protocol, soluble morphogens also may be isolated from one or more body fluids, including serum, cerebro-spinal fluid or
5 peritoneal fluid.

IMAC was performed using Chelating-Sepharose (Pharmacia) that had been charged with three column volumes of 0.2 M ZnSO_4 . The conditioned media was
10 titrated to pH 7.0 and applied directly to the ZN-IMAC resin equilibrated in 20 mM HEPES (pH 7.0) with 500 mM NaCl. The Zn-IMAC resin was loaded with 80 mL of starting conditioned media per mL of resin. After loading the column was washed with equilibration buffer
15 and most of the contaminating proteins were eluted with 35 mM imidazole (pH 7.0) in equilibration buffer. The soluble OP-1 complex is then eluted with 50 mM imidazole (pH 8.0) in 20 mM HEPES and 500 mM NaCl.

20 The 50 mM imidazole eluate containing the soluble OP-1 complex was diluted with nine volumes of 20 mM NaPO_4 (pH 7.0) and applied to an S-Sepharose (Pharmacia) column equilibrated in 20 mM NaPO_4 (pH 7.0) with 50 mM NaCl. The S-Sepharose resin was loaded with
25 an equivalent of 800 mL of starting conditioned media per mL of resin. After loading the S-Sepharose column was washed with equilibration buffer and eluted with 100 mM NaCl followed by 300 mM and 500 mM NaCl in 20 mM NaPO_4 (pH 7.0). The 300 mM NaCl pool was further
30 purified using gel filtration chromatography. Fifty mls of the 300 mM NaCl eluate was applied to a 5.0 X 90 cm Sephacryl S-200HR (Pharmacia) equilibrated in Tris buffered saline (TBS), 50 mM Tris, 150 mM NaCl (pH 7.4). The column was eluted at a flow rate of 5

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mL/minute collecting 10 mL fractions. The apparent molecular of the soluble OP-1 was determined by comparison to protein molecular weight standards (alcohol dehydrogenase (ADH, 150 kDa), bovine serum albumin (BSA, 68 kDa), carbonic anhydrase (CA, 30 kDa) and cytochrome C (cyt C, 12.5 kDa). (see Fig. 3) The purity of the S-200 column fractions was determined by separation on standard 15% polyacrylamide SDS gels stained with coomassie blue. The identity of the mature OP-1 and the pro-domain was determined by N-terminal sequence analysis after separation of the mature OP-1 from the pro-domain using standard reverse phase C18 HPLC.

Figure 3 shows the absorbance profile at 280 nm. The soluble OP-1 complex elutes with an apparent molecular weight of 110 kDa. This agrees well with the predicted composition of the soluble OP-1 complex with one mature OP-1 dimer (35-36 kDa) associated with two pro-domains (39 kDa each). Purity of the final complex can be verified by running the appropriate fraction in a reduced 15% polyacrylamide gel.

The complex components can be verified by running the complex-containing fraction from the S-200 or S-200HR columns over a reverse phase C18 HPLC column and eluting in an acetonitrile gradient (in 0.1% TFA), using standard procedures. The complex is dissociated by this step, and the pro domain and mature species elute as separate species. These separate species then can be subjected to N-terminal sequencing using standard procedures (see, for example, Guide to Protein Purification, M. Deutscher, ed., Academic Press, San Diego, 1990, particularly pp. 602-613), and

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the identity of the isolated 36kD, 39kDa proteins confirmed as mature morphogen and isolated, cleaved pro domain, respectively. N-terminal sequencing of the isolated pro domain from mammalian cell produced OP-1
5 revealed 2 forms of the pro region, the intact form (beginning at residue 30 of Seq. ID No. 1) and a truncated form, (beginning at residue 48 of Seq. ID No. 1.) N-terminal sequencing of the polypeptide subunit of the isolated mature species reveals a range of N-
10 termini for the mature sequence, beginning at residues 293, 300, 313, 315, 316, and 318, of Seq. ID No. 1, all of which are active as demonstrated by the standard bone induction assay.

15 V. In Vitro Soluble Morphogen Complex Formation

As an alternative to purifying soluble complexes from culture media or a body fluid, soluble complexes may be formulated from purified pro domains and mature
20 dimeric species. Successful complex formation apparently requires association of the components under denaturing conditions sufficient to relax the folded structure of these molecules, without affecting disulfide bonds. Preferably, the denaturing conditions
25 mimic the environment of an intracellular vesicle sufficiently such that the cleaved pro domain has an opportunity to associate with the mature dimeric species under relaxed folding conditions. The concentration of denaturant in the solution then is
30 decreased in a controlled, preferably step-wise manner, so as to allow proper refolding of the dimer and pro regions while maintaining the association of the pro

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domain with the dimer. Useful denaturants include 4-6M urea or guanidine hydrochloride (GuHCl), in buffered solutions of pH 4-10, preferably pH 6-8. The soluble complex then is formed by controlled dialysis or
5 dilution into a solution having a final denaturant concentration of less than 0.1-2M urea or GuHCl, preferably 1-2 M urea or GuHCl, which then preferably can be diluted into a physiological buffer. Protein purification/renaturing procedures and considerations
10 are well described in the art, and details for developing a suitable renaturing protocol readily can be determined by one having ordinary skill in the art. One useful text on the subject is Guide to Protein Purification, M. Deutscher, ed., Academic Press, San
15 Diego, 1990, particularly section V. Complex formation also may be aided by addition of one or more chaperone proteins.

VI. Stability of Soluble Morphogen Complexes

20

The stability of the highly purified soluble morphogen complex in a physiological buffer, e.g., tris-buffered saline (TBS) and phosphate-buffered saline (PBS), can be enhanced by any of a number of
25 means. Currently preferred is by means of a pro region that comprises at least the first 18 amino acids of the pro sequence (e.g., residues 30-47 of Seq. ID NO. 1 for OP-1), and preferably is the full length pro region. Residues 30-47 show sequence homology to the N-terminal
30 portion of other morphogens and are believed to have particular utility in enhancing complex stability for

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all morphogens. Other useful means for enhancing the stability of soluble morphogen complexes include three classes of additives. These additives include basic amino acids (e.g., L-arginine, lysine and betaine);
5 nonionic detergents (e.g., Tween 80 or Nonidet P-120); and carrier proteins (e.g., serum albumin and casein). Useful concentrations of these additives include 1-100 mM, preferably 10-70 mM, including 50 mM, basic amino acid;; 0.01-1.0%, preferably 0.05-0.2%, including 0.1%
10 (v/v) nonionic detergent;; and 0.01-1.0%, preferably 0.05-0.2%, including 0.1% (w/v) carrier protein.

VII. Activity of Soluble Morphogen Complex

15 Association of the pro domain with the mature dimeric species does not interfere with the morphogenic activity of the protein in vivo as demonstrated by different activity assays. Specifically, soluble OP-1 complex provided in a standard rat osteopenia model
20 induces significant increase in bone growth and osteocalcin production (see Table II, below), in a manner analogous to the results obtained using mature morphogen.

25 The assay is analogous to the osteoporosis model described in international application US92/07432 (WO93/05751), but uses aged female rats rather than ovariectomized animals. Briefly, young or aged female rats (Charles River Labs, 115-145, and 335-460g body
30 weight, respectively) were dosed daily for 7 days by intravenous tail injection, with either 20 $\mu\text{g/Kg}$ body weight soluble OP-1, or 100 $\mu\text{g/Kg}$ body weight soluble OP-1. Control groups of young and aged female rats were dosed only with tris-buffered saline (TBS). Water

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and food were provided to all animals ad libitum. After 14 days, animals were sacrificed, and new bone growth measured by standard histometric procedures. Osteocalcin concentrations in serum also were measured.

- 5 No detrimental effects of morphogen administration were detected as determined by changes in animal body or organ weight or by hematology profiles.

TABLE II

10	No. Animals	Animal Group	Bone Area (B.Ar/T.Ar)	Osteocalcin (ng/ml)
15	4	Control	5.50 \pm 0.64	11.89 \pm 4.20
20	5	Aged female, 20 μ g/Kg sol. OP-1	7.68 \pm 0.63**	22.24 \pm 2.28**
25	5	Aged female, 100 μ g/Kg sol. OP-1	9.82 \pm 3.31*	20.87 \pm 6.14*

*P < 0.05

**P < 0.01

- 30 Similar experiments performed using soluble OP-1 complex in the osteoporosis model described in WO93/05751 using ovariectomized rats also show no detrimental effect using the complex form.

- 35 Both mature and soluble morphogen also can induce CAM (cell adhesion molecule) expression, as demonstrated below. Briefly, induction of N-CAM isoforms (N-CAM-180, N-CAM-140 and N-CAM-120) can be monitored by reaction with the commercially available
40 antibody mAb H28.123 (Sigma Co., St. Louis) and

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available antibody mAb H28.123 (Sigma Co., St. Louis) and standard Western blot analysis (see, for example, Molecular Cloning, A Laboratory Manual, Sambrook et al. eds. Cold Spring Harbor Press, New York, 1989, particularly Section 18). Incubation of a growing culture of transformed cells of neuronal origin, NG108-15 cells (ATCC, Rockville, MD) with either mature morphogen dimers or soluble morphogen complexes (10-100 ng/ml, preferably at least 40 ng/ml) induces a redifferentiation of these cells back to a morphology characteristic of untransformed neurons, including specific induction and/or enhanced expression of all 3 N-CAM isoforms. In the experiment, cells were subcultured on poly-L-lysine coated 6-well plates and grown in chemically defined medium for 2 days before the experiment. Fresh aliquots of morphogen were added (2.5 μ l) daily.

VIII. Antibody Production

Provided below are standard protocols for polyclonal and monoclonal antibody production. For antibodies which recognize the soluble complex only, preferably the isolated pro region is used as the antigen; where antibodies specific to the mature protein are desired, the antigen preferably comprises at least the C-terminal domain or the intact mature sequence.

Polyclonal antibody may be prepared as follows. Each rabbit is given a primary immunization of 100 ug/500 μ l of antigen, in 0.1% SDS mixed with 500 μ l Complete Freund's Adjuvant. The antigen is injected

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subcutaneously at multiple sites on the back and flanks of the animal. The rabbit is boosted after a month in the same manner using incomplete Freund's Adjuvant. Test bleeds are taken from the ear vein seven days
5 later. Two additional boosts and test bleeds are performed at monthly intervals until antibody against the morphogen antigen is detected in the serum using an ELISA assay. Then, the rabbit is boosted monthly with 100 μ g of antigen and bled (15 ml per bleed) at days
10 seven and ten after boosting.

Monoclonal antibody specific for a given morphogen may be prepared as follows. A mouse is given two injections of the morphogen antigen. The protein or
15 protein fragment preferably is recombinantly produced. The first injection contains 100 μ g of antigen in complete Freund's adjuvant and is given subcutaneously. The second injection contains 50 μ g of antigen in incomplete adjuvant and is given intraperitoneally.
20 The mouse then receives a total of 230 μ g of OP-3 in four intraperitoneal injections at various times over an eight month period. One week prior to fusion, the mouse is boosted intraperitoneally with antigen (e.g., 100 μ g) and may be additionally boosted with a peptide
25 fragment conjugated to bovine serum albumin with a suitable crosslinking agent. This boost can be repeated five days (IP), four days (IP), three days (IP) and one day (IV) prior to fusion. The mouse spleen cells then are fused to commercially available
30 myeloma cells at a ratio of 1:1 using PEG 1500

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(Boeringer Mannheim, Germany), and the fused cells plated and screened for mature or soluble morphogen-specific antibodies using the appropriate portion of the morphogen sequence as antigen. The cell fusion and
5 monoclonal screening steps readily are performed according to standard procedures well described in standard texts widely available in the art.

Using these standard procedures, anti-pro domain
10 antisera was prepared from rabbits using the isolated pro domain from OP-1 as the antigen, and monoclonal antibody ("mAb") to the mature domain was produced in mice, using an E. coli-produced truncated form of OP-1 as antigen.

15

Standard Western blot analysis performed under reducing conditions demonstrates that the anti-pro domain antisera ("anti-pro") is specific for the pro domain only, while the mAb to mature OP-1 ("anti-mature
20 OP-1") is specific for the dimer subunits, that the two antibodies do not cross-react, and that the antibodies and can be used to distinguish between soluble and mature protein forms in a sample, e.g., of conditioned media or serum. A tabular representation of the
25 Western blot results is in Table III below, where reactivity of mAb to mature OP-1 is indicated by "yy", and reactivity of the anti-pro antisera is indicated by "xx".

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TABLE III

	Antibody	Purified Sol OP1	Conditioned CHO Cell Media	Isolated Pro Domain	Purified Dimer Subunits
5	"anti-pro"	xx	xx	xx	
10	"anti-mature OP-1"	yy	yy		yy

15 IX. Immunoassays

20 The ability to detect morphogens in solution and to distinguish between soluble and mature dimeric morphogen forms provides a valuable tool for diagnostic assays, allowing one to monitor the level and type of morphogen free in the body, e.g., in serum and other body fluids, as well as to develop diagnostic and other tissue evaluating kits.

25 For example, OP-1 is an intimate participant in normal bone growth and resorption. Thus, soluble OP-1 is expected to be detected at higher concentrations in individuals experiencing high bone turnover, such as children, and at substantially lower levels in
 30 individuals with abnormally low rates of bone turnover, such as patients with osteoporosis, osteosarcoma, Paget's disease and the like. Monitoring the level of OP-1, or other bone targeted morphogens such as BMP2 and BMP4, in serum thus provides a means for evaluating
 35 the status of bone tissue in an individual, as well as a means for monitoring the efficacy of a treatment to regenerate damaged or lost bone tissue. Similarly,

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monitoring the level of endogenous GDF-1, can provide diagnostic information on the health of nerve tissue, particularly brain tissue. Moreover, following this disclosure one can distinguish between the level of
5 soluble and mature forms in solution.

A currently preferred detection means for evaluating the level of morphogen in a body fluid comprises an immunoassay utilizing an antibody or other
10 suitable binding protein capable of reacting specifically with a morphogen and being detected as part of a complex with the morphogen. Immunoassays may be performed using standard techniques known in the art and antibodies raised against a morphogen and specific
15 for that morphogen. Antibodies which recognize a morphogen protein form of interest may be generated as described herein and these antibodies then used to monitor endogenous levels of protein in a body fluid, such as serum, whole blood or peritoneal fluid. To
20 monitor endogenous concentrations of soluble morphogen, the antibody chosen preferably has binding specificity for the soluble form e.g., has specificity for the pro domain. Such antibodies may be generated by using the pro domain or a portion thereof as the antigen,
25 essentially as described herein. A suitable pro domain for use as an antigen may be obtained by isolating the soluble complex and then separating the noncovalently associated pro domain from the mature domain using standard procedures, e.g., by passing the complex over
30 an HPLC column, as described above or by separation by gel electrophoresis. Alternatively, the pro form of the protein in its monomeric form may be used as the

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antigen and the candidate antibodies screened by Western blot or other standard immunoassay for those which recognize the pro domain of the soluble form of the protein of interest, but not the mature form, also
5 as described above.

Monomeric pro forms can be obtained from cell lysates of CHO produced cells, or from prokaryotic expression of a DNA encoding the pro form, in for
10 example, E.coli. The pro form, which has an apparent molecular weight of about 50 kDa in mammalian cells, can then be isolated by HPLC and/or by gel electrophoresis, as described above.

15 In order to detect and/or quantitate the amount of morphogenic protein present in a solution, an immunoassay may be performed to detect the morphogen using a polyclonal or monoclonal antibody specific for that protein. Here, soluble and mature forms of the
20 morphogen also may be distinguished by using antibodies that discriminate between the two forms of the proteins as described above. Currently preferred assays include ELISAS and radioimmunoassays, including standard competitor assays useful for quantitating the morphogen
25 in a sample, where an unknown amount of sample morphogen is allowed to react with anti-morphogen antibody and this interaction is competed with a known amount of labeled antigen. The level of bound or free labeled antigen at equilibrium then is measured to
30 quantitate the amount of unlabeled antigen in solution, the amount of sample antigen being proportional to the amount of free labeled antigen. Exemplary protocols for these assays are provided below. However, as will be appreciated by those skilled in the art, variations

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of these protocols, as well as other immunoassays, are well known in the literature and within the skill of the art. For example, in the ELISA protocol provided below, soluble OP-1 is identified in a sample using
5 biotinylated anti-pro antiserum. Biotinylated antibodies can be visualized in a colormetric assay or in a chemiluminescent assay, as described below. Alternatively, the antibody can be radio-labeled with a suitable molecule, such as ^{125}I . Still another
10 protocol that may be used is a solid phase immunoassay, preferably using an affinity column with anti-morphogen antibody complexed to the matrix surface and over which a serum sample may be passed. A detailed description of useful immunoassays, including protocols and general
15 considerations is provided in, for example, Molecular Cloning: A Laboratory Manual, Sambrook et al., eds. Cold Spring Harbor Press, New York, 1989, particularly Section 18.

20 For serum assays, the serum preferably first is partially purified to remove some of the excess, contaminating serum proteins, such as serum albumin. Preferably the serum is extracted by precipitation in ammonium sulfate (e.g., 45%) such that the complex is
25 precipitated. Further purification can be achieved using purification strategies that take advantage of the differential solubility of soluble morphogen complex or mature morphogens relative to that of the other proteins present in serum. Further purification
30 also can be achieved by chromatographic techniques well known in the art.

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Soluble OP-1 may be detected using a polyclonal antibody specific for the OP-1 pro domain in an ELISA, as follows. 1 μ g/100 μ l of affinity-purified polyclonal rabbit IgG specific for OP-1-pro is added to each well of a 96-well plate and incubated at 37°C for an hour. The wells are washed four times with 0.167M sodium borate buffer with 0.15 M NaCl (BSB), pH 8.2, containing 0.1% Tween 20. To minimize non-specific binding, the wells are blocked by filling completely with 1% bovine serum albumin (BSA) in BSB and incubating for 1 hour at 37°C. The wells are then washed four times with BSB containing 0.1% Tween 20. A 100 μ l aliquot of an appropriate dilution of each of the test samples of cell culture supernatant or serum sample is added to each well in triplicate and incubated at 37°C for 30 min. After incubation, 100 μ l biotinylated rabbit anti-pro serum (stock solution is about 1 mg/ml and diluted 1:400 in BSB containing 1% BSA before use) is added to each well and incubated at 37°C for 30 min. The wells are then washed four times with BSB containing 0.1% Tween 20. 100 μ l streptavidin-alkaline (Southern Biotechnology Associates, Inc. Birmingham, Alabama, diluted 1:2000 in BSB containing 0.1% Tween 20 before use) is added to each well and incubated at 37°C for 30 min. The plates are washed four times with 0.5M Tris buffered Saline (TBS), pH 7.2. 50 μ l substrate (ELISA Amplification System Kit, Life Technologies, Inc., Bethesda, MD) is added to each well incubated at room temperature for 15 min. Then, 50 μ l amplifier (from the same amplification system kit) is added and incubated for another 15 min at room temperature. The reaction is stopped by the addition of 50 μ l 0.3 M sulphuric acid.

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The OD at 490 nm of the solution in each well is recorded. To quantitate the level of soluble OP-1 in the sample, a standard curve is performed in parallel with the test samples. In the standard curve, known
5 increasing amounts of purified OP-1-pro is added. Alternatively, using, for example, Lumi-phos 530 (Analytical Luminescence Laboratories) as the substrate and detection at 300-650 nm in a standard luminometer, complexes can be detected by chemiluminescence, which
10 typically provides a more sensitive assay than detection by means of a visible color change.

Morphogen (soluble or mature form) may be detected in a standard plated-based radioimmunoassay as follows.
15 Empirically determined limiting levels of anti-morphogen antibody (e.g., anti-OP-1, typically 50-80 ng/well) are bound to wells of a PVC plate e.g., in 50 μ l PBS phosphate buffered saline. After sufficient incubation to allow binding at room
20 temperature, typically one hour, the plate is washed in a PBS/Tween 20 solution, ("washing buffer"), and 200 μ l of block (3% BSA, 0.1 μ lysine in 1xBSB) is added to each well and allowed to incubate for 1 hour, after which the wells are washed again in washing buffer. 40
25 μ l of a sample composed of serially diluted plasma (preferably partially purified as described above) or morphogen standard (e.g., OP-1) is added to wells in triplicate. Samples preferably are diluted in PTTH (15 mM KH_2PO_4 , 8 mM Na_2PO_4 , 27 mM KCl, 137 mM NaCl,
30 0.05% Tween 20, 1 mg/ml HSA, 0.05% NaN_3 , pH 7.2). 10 μ l of labelled competitor antigen, preferably 100,000-500,000 cpm/sample is added (e.g., ^{125}I OP-1, radiolabelled using standard procedures), and plates are incubated overnight at 4°C. Plates then are washed

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in washing buffer, and allowed to dry. Wells are cut apart and bound labelled OP-1 counted in a standard gamma counter. The quantities of bound labelled antigen (e.g., ^{125}I OP-1) measured in the presence and
5 absence of sample then are compared, the difference being proportional to the amount of sample antigen (morphogen) present in the sample fluid.

As a corollary assay method, immunoassays may be
10 developed to detect endogenous anti-morphogen antibodies, and to distinguish between such antibodies to soluble or mature forms. Endogenous anti-morphogen antibodies have been detected in serum, and their level is known to increase, for example, upon implanting of
15 an osteogenic device in a mammal. Without being limited to a particular theory, these antibodies may play a role in modulating morphogen activity by modulating the level of available protein in serum. Assays that monitor the level of endogenous antibodies
20 in blood or their body fluids thus can be used in diagnostic assays to evaluate the status of a tissue, as well as to provide a means for monitoring the efficacy of a therapy for tissue regeneration.

25 The currently preferred means for detecting endogenous anti-morphogen antibodies is by means of a standard Western blot. See, for example, Molecular Cloning: A Laboratory Manual Sambrook et al., eds., Cold Spring Harbor Press, New York, 1989, particularly
30 pages 18.60-18.75, incorporated herein by reference, for a detailed description of these assays. Purified mature or soluble morphogen is electrophoresed on an SDS polyacrylamide gel under oxidized or reduced conditions designed to separate the proteins in

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solution, and the proteins then transferred to a polyvinylidene difluoride microporus membrane (0.45 μ m pore sizes) using standard buffers and procedures. The filter then is incubated with the
5 serum being tested (at various dilutions). Antibodies bound to either the pro domain or the mature morphogen domain are detected by means of an anti-human antibody protein, e.g., goat anti-human Ig. Titers of the antimorphogen antibodies can be determined by further
10 dilution of the serum until no signal is detected.

X. Formulations and Methods for Administering Soluble Morphogens as Therapeutic Agents

15 The soluble morphogens of this invention are particularly useful as therapeutic agents to regenerate diseased or damaged tissue in a mammal, particularly a human.

20 The soluble morphogen complexes may be used to particular advantage in regeneration of damaged or diseased lung, heart, liver, kidney, nerve or pancreas tissue, as well as in the transplantation and/or grafting of these tissues and bone marrow, skin,
25 gastrointestinal mucosa, and other living tissues.

The soluble morphogen complexes described herein may be provided to an individual by any suitable means, preferably directly or systemically, e.g., parenterally
30 or orally. Where the morphogen is to be provided directly (e.g., locally, as by injection, to a desired tissue site), or parenterally, such as by intravenous, subcutaneous, intramuscular, intraorbital, ophthalmic, intraventricular, intracranial, intracapsular,

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intraspinal, intracisternal, intraperitoneal, buccal, rectal, vaginal, intranasal or by aerosol administration, the soluble morphogen complex preferably comprises part of an aqueous solution. The
5 solution is physiologically acceptable so that in addition to delivery of the desired morphogen to the patient, the solution does not otherwise adversely affect the patient's electrolyte and volume balance. The aqueous medium for the soluble morphogen thus may
10 comprise normal physiologic saline (0.9% NaCl, 0.15M), pH 7-7.4.

Soluble morphogens of this invention are readily purified from cultured cell media into a physiological
15 buffer, as described above. In addition, and as described above, if desired, the soluble complexes may be formulated with one or more additional additives, including basic amino acids (e.g., L-arginine, lysine, betaine); non-ionic detergents (e.g. Tween-80 or
20 Nonidet-120) and carrier proteins (e.g., serum albumin and casein).

Useful solutions for oral or parenteral administration may be prepared by any of the methods
25 well known in the pharmaceutical art, described, for example, in Remington's Pharmaceutical Sciences, (Gennaro, A., ed.), Mack Pub., 1990. Formulations may include, for example, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin,
30 hydrogenated naphthalenes, and the like. Formulations for direct administration, in particular, may include glycerol and other compositions of high viscosity.

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Biocompatible, preferably bioresorbable polymers, including, for example, hyaluronic acid, collagen, tricalcium phosphate, polybutyrate, polylactide, polyglycolide and lactide/glycolide copolymers, may be
5 useful excipients to control the release of the soluble morphogen in vivo.

Other potentially useful parenteral delivery systems for these morphogens include ethylene-vinyl
10 acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation administration may contain as excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl
15 ether, glycocholate and deoxycholate, or oily solutions for administration in the form of nasal drops, or as a gel to be applied intranasally.

The soluble morphogens described herein also may be
20 administered orally. Oral administration of proteins as therapeutics generally is not practiced as most proteins readily are degraded by digestive enzymes and acids in the mammalian digestive system before they can be absorbed into the bloodstream. However, the mature
25 domains of the morphogens described herein typically are acid-stable and protease-resistant (see, for example, U.S. Pat. No. 4,968,590.) In addition, at least one morphogen, OP-1, has been identified, in mammary gland extract, colostrum and milk, as well as
30 saliva. Moreover, the OP-1 purified from mammary gland extract is morphogenically active. For example, this protein induces endochondral bone formation in mammals when implanted subcutaneously in association with a suitable matrix material, using a standard in vivo bone

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assay, such as is disclosed in U.S. Pat. No. 4,968,590. In addition, endogenous morphogen also is detected in human serum (see above). Finally, comparative experiments with soluble and mature morphogens in a
5 number of experiments defining morphogenic activity indicate that the non-covalent association of the pro domain with the dimeric species does not interfere with morphogenic activity. These findings indicate that oral and parenteral administration are viable means for
10 administering morphogens to an individual, and that soluble morphogens have utility in systemic administration protocols.

The soluble complexes provided herein also may be
15 associated with molecules capable of targeting the morphogen to a desired tissue. For example, tetracycline and diphosphonates (bisphosphonates) are known to bind to bone mineral, particularly at zones of bone remodeling, when they are provided systemically in
20 a mammal. Accordingly, these molecules may be included as useful agents for targeting soluble morphogens to bone tissue. Alternatively, an antibody or other binding protein that interacts specifically with a surface molecule on the desired target tissue cells
25 also may be used. Such targeting molecules further may be covalently associated to the morphogen complex, e.g., by chemical crosslinking, or by using standard genetic engineering means to create, for example, an acid labile bond such as an Asp-Pro linkage. Useful
30 targeting molecules may be designed, for example, using the single chain binding site technology disclosed, for example, in U.S. Pat. No. 5,091,513.

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Finally, the soluble morphogen complexes provided herein may be administered alone or in combination with other molecules known to have a beneficial effect on tissue morphogenesis, including molecules capable of
5 tissue repair and regeneration and/or inhibiting inflammation. Examples of useful cofactors for stimulating bone tissue growth in osteoporotic individuals, for example, include but are not limited to, vitamin D₃, calcitonin, prostaglandins, parathyroid
10 hormone, dexamethasone, estrogen and IGF-I or IGF-II. Useful cofactors for nerve tissue repair and regeneration may include nerve growth factors. Other useful cofactors include symptom-alleviating cofactors, including antiseptics, antibiotics, antiviral and
15 antifungal agents and analgesics and anesthetics.

The compounds provided herein can be formulated into pharmaceutical compositions by admixture with pharmaceutically acceptable nontoxic excipients and
20 carriers. As noted above, such compositions may be prepared for parenteral administration, particularly in the form of liquid solutions or suspensions; for oral administration, particularly in the form of tablets or capsules; or intranasally, particularly in the form of
25 powders, nasal drops or aerosols. Where adhesion to a tissue surface is desired the composition may include the morphogen dispersed in a fibrinogen-thrombin composition or other bioadhesive such as is disclosed, for example in PCT US91/09275, the disclosure of which
30 is incorporated herein by reference. The composition then may be painted, sprayed or otherwise applied to the desired tissue surface.

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The compositions can be formulated for parenteral or oral administration to humans or other mammals in therapeutically effective amounts, e.g., amounts which provide appropriate concentrations of the morphogen to target tissue for a time sufficient to induce morphogenesis, including particular steps thereof, as described above.

Where the soluble morphogen complex is to be used as part of a transplant procedure, the morphogen may be provided to the living tissue or organ to be transplanted prior to removal of the tissue or organ from the donor. The morphogen may be provided to the donor host directly, as by injection of a formulation comprising the soluble complex into the tissue, or indirectly, e.g., by oral or parenteral administration, using any of the means described above.

Alternatively or, in addition, once removed from the donor, the organ or living tissue may be placed in a preservation solution containing the morphogen. In addition, the recipient also preferably is provided with the morphogen just prior to, or concomitant with, transplantation. In all cases, the soluble complex may be administered directly to the tissue at risk, as by injection to the tissue, or it may be provided systemically, either by oral or parenteral administration, using any of the methods and formulations described herein and/or known in the art.

Where the morphogen comprises part of a tissue or organ preservation solution, any commercially available preservation solution may be used to advantage. A

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useful preservation solution is described in in PCT/US92/07358 (WO93/04692), incorporated herein by reference.

5 As will be appreciated by those skilled in the art, the concentration of the compounds described in a therapeutic composition will vary depending upon a number of factors, including the dosage of the drug to be administered, the chemical characteristics (e.g.,
10 hydrophobicity) of the compounds employed, and the route of administration. The preferred dosage of drug to be administered also is likely to depend on such variables as the type and extent of tissue loss or defect, the overall health status of the particular
15 patient, the relative biological efficacy of the compound selected, the formulation of the compound, the presence and types of excipients in the formulation, and the route of administration. In general terms, the compounds of this invention may be provided in an
20 aqueous physiological buffer solution containing about 0.001 to 10% w/v compound for parenteral administration. Typical dose ranges are from about 10 ng/kg to about 1 g/kg of body weight per day; a preferred dose range is from about 0.1 μ g/kg to
25 100 mg/kg of body weight. No obvious morphogen-induced pathological lesions are induced when mature morphogen (e.g., OP-1, 20 μ g) is administered daily to normal growing rats for 21 consecutive days. Moreover, 10 μ g systemic injections of morphogen (e.g., OP-1) injected
30 daily for 10 days into normal newborn mice does not produce any gross abnormalities.

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Where morphogens are administered systemically, in the methods of the present invention, preferably a large volume loading dose is used at the start of the treatment. The treatment then is continued with a
5 maintenance dose. Further administration then can be determined by monitoring at intervals the levels of the morphogen in the blood.

Other Embodiments

10

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The present embodiments are therefore to be considered in all respects as
15 illustrative and not restrictive, the scope of the invention being indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced
20 therein.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

5

(i) APPLICANT:

- (A) NAME: CREATIVE BIOMOLECULES, INC.
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- (H) TELEFAX: 1-508-435-0454
- 15 (I) TELEX:

(ii) TITLE OF INVENTION: NOVEL MORPHOGENIC PROTEIN COMPOSITIONS
OF MATTER

20

(iii) NUMBER OF SEQUENCES: 23

(iv) CORRESPONDENCE ADDRESS:

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- 30 (F) ZIP: 01748

(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- 35 (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER:
- (B) FILING DATE:
- 40 (C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER:
- (B) FILING DATE:
- 45

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: KELLEY, ROBIN, D.
- (B) REGISTRATION NUMBER: 34,637
- 50 (C) REFERENCE/DOCKET NUMBER: CRP-081CP

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1822 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) ORIGINAL SOURCE:
 (A) ORGANISM: HOMO SAPIENS
 (F) TISSUE TYPE: HIPPOCAMPUS

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 49..1341
 (C) IDENTIFICATION METHOD: experimental
 (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
 /product= "OP1"
 /evidence= EXPERIMENTAL
 /standard_name= "OP1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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											Met	His	Val				
											1						
35	CGC	TCA	CTG	CGA	GCT	GCG	GCG	CCG	CAC	AGC	TTC	GTG	GCG	CTC	TGG	GCA	105
	Arg	Ser	Leu	Arg	Ala	Ala	Ala	Pro	His	Ser	Phe	Val	Ala	Leu	Trp	Ala	
	5					10					15						
40	CCC	CTG	TTC	CTG	CTG	CGC	TCC	GCC	CTG	GCC	GAC	TTC	AGC	CTG	GAC	AAC	153
	Pro	Leu	Phe	Leu	Leu	Arg	Ser	Ala	Leu	Ala	Asp	Phe	Ser	Leu	Asp	Asn	
	20					25					30					35	
45	GAG	GTG	CAC	TCG	AGC	TTC	ATC	CAC	CGG	CGC	CTC	CGC	AGC	CAG	GAG	CGG	201
	Glu	Val	His	Ser	Ser	Phe	Ile	His	Arg	Arg	Leu	Arg	Ser	Gln	Glu	Arg	
				40					45					50			
	CGG	GAG	ATG	CAG	CGC	GAG	ATC	CTC	TCC	ATT	TTG	GGC	TTG	CCC	CAC	CGC	249
	Arg	Glu	Met	Gln	Arg	Glu	Ile	Leu	Ser	Ile	Leu	Gly	Leu	Pro	His	Arg	
				55					60					65			

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	CCG	CGC	CCG	CAC	CTC	CAG	GGC	AAG	CAC	AAC	TCG	GCA	CCC	ATG	TTC	ATG	297
	Pro	Arg	Pro	His	Leu	Gln	Gly	Lys	His	Asn	Ser	Ala	Pro	Met	Phe	Met	
			70					75					80				
5	CTG	GAC	CTG	TAC	AAC	GCC	ATG	GCG	GTG	GAG	GAG	GGC	GGC	GGG	CCC	GGC	345
	Leu	Asp	Leu	Tyr	Asn	Ala	Met	Ala	Val	Glu	Glu	Gly	Gly	Gly	Pro	Gly	
		85					90					95					
10	GGC	CAG	GGC	TTC	TCC	TAC	CCC	TAC	AAG	GCC	GTC	TTC	AGT	ACC	CAG	GGC	393
	Gly	Gln	Gly	Phe	Ser	Tyr	Pro	Tyr	Lys	Ala	Val	Phe	Ser	Thr	Gln	Gly	
	100					105					110					115	
15	CCC	CCT	CTG	GCC	AGC	CTG	CAA	GAT	AGC	CAT	TTC	CTC	ACC	GAC	GCC	GAC	441
	Pro	Pro	Leu	Ala	Ser	Leu	Gln	Asp	Ser	His	Phe	Leu	Thr	Asp	Ala	Asp	
					120					125					130		
20	ATG	GTC	ATG	AGC	TTC	GTC	AAC	CTC	GTG	GAA	CAT	GAC	AAG	GAA	TTC	TTC	489
	Met	Val	Met	Ser	Phe	Val	Asn	Leu	Val	Glu	His	Asp	Lys	Glu	Phe	Phe	
				135					140					145			
25	CAC	CCA	CGC	TAC	CAC	CAT	CGA	GAG	TTC	CGG	TTT	GAT	CTT	TCC	AAG	ATC	537
	His	Pro	Arg	Tyr	His	His	Arg	Glu	Phe	Arg	Phe	Asp	Leu	Ser	Lys	Ile	
			150					155					160				
30	CCA	GAA	GGG	GAA	GCT	GTC	ACG	GCA	GCC	GAA	TTC	CGG	ATC	TAC	AAG	GAC	585
	Pro	Glu	Gly	Glu	Ala	Val	Thr	Ala	Ala	Glu	Phe	Arg	Ile	Tyr	Lys	Asp	
		165					170					175					
35	TAC	ATC	CGG	GAA	CGC	TTC	GAC	AAT	GAG	ACG	TTC	CGG	ATC	AGC	GTT	TAT	633
	Tyr	Ile	Arg	Glu	Arg	Phe	Asp	Asn	Glu	Thr	Phe	Arg	Ile	Ser	Val	Tyr	
	180					185					190					195	
40	CAG	GTG	CTC	CAG	GAG	CAC	TTG	GGC	AGG	GAA	TCG	GAT	CTC	TTC	CTG	CTC	681
	Gln	Val	Leu	Gln	Glu	His	Leu	Gly	Arg	Glu	Ser	Asp	Leu	Phe	Leu	Leu	
				200						205					210		
45	GAC	AGC	CGT	ACC	CTC	TGG	GCC	TCG	GAG	GAG	GGC	TGG	CTG	GTG	TTT	GAC	729
	Asp	Ser	Arg	Thr	Leu	Trp	Ala	Ser	Glu	Glu	Gly	Trp	Leu	Val	Phe	Asp	
				215					220					225			
50	ATC	ACA	GCC	ACC	AGC	AAC	CAC	TGG	GTG	GTC	AAT	CCG	CGG	CAC	AAC	CTG	777
	Ile	Thr	Ala	Thr	Ser	Asn	His	Trp	Val	Val	Asn	Pro	Arg	His	Asn	Leu	
			230					235					240				
55	GGC	CTG	CAG	CTC	TCG	GTG	GAG	ACG	CTG	GAT	GGG	CAG	AGC	ATC	AAC	CCC	825
	Gly	Leu	Gln	Leu	Ser	Val	Glu	Thr	Leu	Asp	Gly	Gln	Ser	Ile	Asn	Pro	
		245					250					255					

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	AAG TTG GCG GGC CTG ATT GGG CGG CAC GGG CCC CAG AAC AAG CAG CCC	873
	Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro	
	260 265 270 275	
5	TTC ATG GTG GCT TTC TTC AAG GCC ACG GAG GTC CAC TTC CGC AGC ATC	921
	Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe Arg Ser Ile	
	280 285 290	
10	CGG TCC ACG GGG AGC AAA CAG CGC AGC CAG AAC CGC TCC AAG ACG CCC	969
	Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro	
	295 300 305	
15	AAG AAC CAG GAA GCC CTG CGG ATG GCC AAC GTG GCA GAG AAC AGC AGC	1017
	Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser	
	310 315 320	
20	AGC GAC CAG AGG CAG GCC TGT AAG AAG CAC GAG CTG TAT GTC AGC TTC	1065
	Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe	
	325 330 335	
25	CGA GAC CTG GGC TGG CAG GAC TGG ATC ATC GCG CCT GAA GGC TAC GCC	1113
	Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala	
	340 345 350 355	
30	GCC TAC TAC TGT GAG GGG GAG TGT GCC TTC CCT CTG AAC TCC TAC ATG	1161
	Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met	
	360 365 370	
35	AAC GCC ACC AAC CAC GCC ATC GTG CAG ACG CTG GTC CAC TTC ATC AAC	1209
	Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn	
	375 380 385	
40	CCG GAA ACG GTG CCC AAG CCC TGC TGT GCG CCC ACG CAG CTC AAT GCC	1257
	Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala	
	390 395 400	
45	ATC TCC GTC CTC TAC TTC GAT GAC AGC TCC AAC GTC ATC CTG AAG AAA	1305
	Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys	
	405 410 415	
50	TAC AGA AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCCTCC	1351
	Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His	
	420 425 430	
45	GAGAATTCAG ACCCTTTGGG GCCAAGTTTT TCTGGATCCT CCATTGCTCG CCTTGGCCAG	1411
	GAACCAGCAG ACCAACTGCC TTTTGTGAGA CCTTCCCCTC CCTATCCCCA ACTTTAAAGG	1471
	TGTGAGAGTA TTAGGAAACA TGAGCAGCAT ATGGCTTTTG ATCAGTTTTT CAGTGGCAGC	1531

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ATCCAATGAA CAAGATCCTA CAAGCTGTGC AGGCAAAACC TAGCAGGAAA AAAAAACAAC 1591
 GCATAAAGAA AAATGGCCGG GCCAGGTCAT TGGCTGGGAA GTCTCAGCCA TGCACGGACT 1651
 5 CGTTTCCAGA GGTAATTATG AGCGCCTACC AGCCAGGCCA CCCAGCCGTG GGAGGAAGGG 1711
 GGCCTGGCAA GGGGTGGGCA CATTGGTGTC TGTGCGAAAG GAAAATTGAC CCGGAAGTTC 1771
 CTGTAATAAA TGTCACAATA AAACGAATGA ATGAAAAAAAA AAAAAAAAAA A 1822
 10

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
 15 (A) LENGTH: 431 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
 20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala
 1 5 10 15
 25 Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser
 20 25 30
 30 Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser
 35 40 45
 Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu
 50 55 60
 35 Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
 65 70 75 80
 Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly
 85 90 95
 40 Gly Pro Gly Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser
 100 105 110
 45 Thr Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr
 115 120 125
 Asp Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys
 130 135 140

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	Glu	Phe	Phe	His	Pro	Arg	Tyr	His	His	Arg	Glu	Phe	Arg	Phe	Asp	Leu	
	145					150					155					160	
5	Ser	Lys	Ile	Pro	Glu	Gly	Glu	Ala	Val	Thr	Ala	Ala	Glu	Phe	Arg	Ile	
					165					170					175		
	Tyr	Lys	Asp	Tyr	Ile	Arg	Glu	Arg	Phe	Asp	Asn	Glu	Thr	Phe	Arg	Ile	
				180					185					190			
10	Ser	Val	Tyr	Gln	Val	Leu	Gln	Glu	His	Leu	Gly	Arg	Glu	Ser	Asp	Leu	
			195					200					205				
	Phe	Leu	Leu	Asp	Ser	Arg	Thr	Leu	Trp	Ala	Ser	Glu	Glu	Gly	Trp	Leu	
	210						215					220					
15	Val	Phe	Asp	Ile	Thr	Ala	Thr	Ser	Asn	His	Trp	Val	Val	Asn	Pro	Arg	
	225					230					235					240	
	His	Asn	Leu	Gly	Leu	Gln	Leu	Ser	Val	Glu	Thr	Leu	Asp	Gly	Gln	Ser	
20				245						250					255		
	Ile	Asn	Pro	Lys	Leu	Ala	Gly	Leu	Ile	Gly	Arg	His	Gly	Pro	Gln	Asn	
				260					265					270			
25	Lys	Gln	Pro	Phe	Met	Val	Ala	Phe	Phe	Lys	Ala	Thr	Glu	Val	His	Phe	
			275					280					285				
	Arg	Ser	Ile	Arg	Ser	Thr	Gly	Ser	Lys	Gln	Arg	Ser	Gln	Asn	Arg	Ser	
	290						295					300					
30	Lys	Thr	Pro	Lys	Asn	Gln	Glu	Ala	Leu	Arg	Met	Ala	Asn	Val	Ala	Glu	
	305					310					315					320	
	Asn	Ser	Ser	Ser	Asp	Gln	Arg	Gln	Ala	Cys	Lys	Lys	His	Glu	Leu	Tyr	
35				325						330					335		
	Val	Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln	Asp	Trp	Ile	Ile	Ala	Pro	Glu	
				340					345					350			
40	Gly	Tyr	Ala	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala	Phe	Pro	Leu	Asn	
			355					360					365				
	Ser	Tyr	Met	Asn	Ala	Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu	Val	His	
	370						375					380					
45	Phe	Ile	Asn	Pro	Glu	Thr	Val	Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln	
	385					390					395					400	

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Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile
405 410 415

Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
5 420 425 430

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 1873 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

20 (A) NAME/KEY: CDS
(B) LOCATION: 104..1393
(D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
/product= "MOP1"
/note= "MOP1 CDNA"

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTGCAGCAAG TGACCTCGGG TCGTGGACCG CTGCCCTGCC CCCTCCGCTG CCACCTGGGG 60

30 CGGCGCGGGC CCGGTGCCCC GGATCGCGCG TAGAGCCGGC GCG ATG CAC GTG CGC 115
Met His Val Arg
1

	TCG	CTG	CGC	GCT	GCG	GCG	CCA	CAC	AGC	TTC	GTG	GCG	CTC	TGG	GCG	CCT	163
35	Ser	Leu	Arg	Ala	Ala	Ala	Pro	His	Ser	Phe	Val	Ala	Leu	Trp	Ala	Pro	
	5					10					15					20	

40 CTG TTC TTG CTG CGC TCC GCC CTG GCC GAT TTC AGC CTG GAC AAC GAG 211
Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn Glu
25 30 35

GTG CAC TCC AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG CGG 259
Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg Arg
40 45 50

45 GAG ATG CAG CGG GAG ATC CTG TCC ATC TTA GGG TTG CCC CAT CGC CCG 307
Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg Pro
55 60 65

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	CGC	CCG	CAC	CTC	CAG	GGA	AAG	CAT	AAT	TCG	GCG	CCC	ATG	TTC	ATG	TTG	355
	Arg	Pro	His	Leu	Gln	Gly	Lys	His	Asn	Ser	Ala	Pro	Met	Phe	Met	Leu	
	70					75					80						
5	GAC	CTG	TAC	AAC	GCC	ATG	GCG	GTG	GAG	GAG	AGC	GGG	CCG	GAC	GGA	CAG	403
	Asp	Leu	Tyr	Asn	Ala	Met	Ala	Val	Glu	Glu	Ser	Gly	Pro	Asp	Gly	Gln	
	85				90				95						100		
10	GGC	TTC	TCC	TAC	CCC	TAC	AAG	GCC	GTC	TTC	AGT	ACC	CAG	GGC	CCC	CCT	451
	Gly	Phe	Ser	Tyr	Pro	Tyr	Lys	Ala	Val	Phe	Ser	Thr	Gln	Gly	Pro	Pro	
					105				110						115		
15	TTA	GCC	AGC	CTG	CAG	GAC	AGC	CAT	TTC	CTC	ACT	GAC	GCC	GAC	ATG	GTC	499
	Leu	Ala	Ser	Leu	Gln	Asp	Ser	His	Phe	Leu	Thr	Asp	Ala	Asp	Met	Val	
				120					125					130			
20	ATG	AGC	TTC	GTC	AAC	CTA	GTG	GAA	CAT	GAC	AAA	GAA	TTC	TTC	CAC	CCT	547
	Met	Ser	Phe	Val	Asn	Leu	Val	Glu	His	Asp	Lys	Glu	Phe	Phe	His	Pro	
			135					140					145				
25	CGA	TAC	CAC	CAT	CGG	GAG	TTC	CGG	TTT	GAT	CTT	TCC	AAG	ATC	CCC	GAG	595
	Arg	Tyr	His	His	Arg	Glu	Phe	Arg	Phe	Asp	Leu	Ser	Lys	Ile	Pro	Glu	
	150					155					160						
30	GGC	GAA	CGG	GTG	ACC	GCA	GCC	GAA	TTC	AGG	ATC	TAT	AAG	GAC	TAC	ATC	643
	Gly	Glu	Arg	Val	Thr	Ala	Ala	Glu	Phe	Arg	Ile	Tyr	Lys	Asp	Tyr	Ile	
	165					170				175						180	
35	CGG	GAG	CGA	TTT	GAC	AAC	GAG	ACC	TTC	CAG	ATC	ACA	GTC	TAT	CAG	GTG	691
	Arg	Glu	Arg	Phe	Asp	Asn	Glu	Thr	Phe	Gln	Ile	Thr	Val	Tyr	Gln	Val	
				185					190						195		
40	CTC	CAG	GAG	CAC	TCA	GGC	AGG	GAG	TCG	GAC	CTC	TTC	TTG	CTG	GAC	AGC	739
	Leu	Gln	Glu	His	Ser	Gly	Arg	Glu	Ser	Asp	Leu	Phe	Leu	Leu	Asp	Ser	
				200					205					210			
45	CGC	ACC	ATC	TGG	GCT	TCT	GAG	GAG	GGC	TGG	TTG	GTG	TTT	GAT	ATC	ACA	787
	Arg	Thr	Ile	Trp	Ala	Ser	Glu	Glu	Gly	Trp	Leu	Val	Phe	Asp	Ile	Thr	
			215				220						225				
50	GCC	ACC	AGC	AAC	CAC	TGG	GTG	GTC	AAC	CCT	CGG	CAC	AAC	CTG	GGC	TTA	835
	Ala	Thr	Ser	Asn	His	Trp	Val	Val	Asn	Pro	Arg	His	Asn	Leu	Gly	Leu	
	230					235						240					
55	CAG	CTC	TCT	GTG	GAG	ACC	CTG	GAT	GGG	CAG	AGC	ATC	AAC	CCC	AAG	TTG	883
	Gln	Leu	Ser	Val	Glu	Thr	Leu	Asp	Gly	Gln	Ser	Ile	Asn	Pro	Lys	Leu	
	245					250				255						260	

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	GCA GGC CTG ATT GGA CGG CAT GGA CCC CAG AAC AAG CAA CCC TTC ATG	931
	Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro Phe Met	
	265 270 275	
5	GTG GCC TTC TTC AAG GCC ACG GAA GTC CAT CTC CGT AGT ATC CGG TCC	979
	Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg Ser Ile Arg Ser	
	280 285 290	
10	ACG GGG GGC AAG CAG CGC AGC CAG AAT CGC TCC AAG ACG CCA AAG AAC	1027
	Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys Asn	
	295 300 305	
15	CAA GAG GCC CTG AGG ATG GCC AGT GTG GCA GAA AAC AGC AGC AGT GAC	1075
	Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn Ser Ser Ser Asp	
	310 315 320	
20	CAG AGG CAG GCC TGC AAG AAA CAT GAG CTG TAC GTC AGC TTC CGA GAC	1123
	Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp	
	325 330 335 340	
25	CTT GGC TGG CAG GAC TGG ATC ATT GCA CCT GAA GGC TAT GCT GCC TAC	1171
	Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr	
	345 350 355	
30	TAC TGT GAG GGA GAG TGC GCC TTC CCT CTG AAC TCC TAC ATG AAC GCC	1219
	Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn Ala	
	360 365 370	
35	ACC AAC CAC GCC ATC GTC CAG ACA CTG GTT CAC TTC ATC AAC CCA GAC	1267
	Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Asp	
	375 380 385	
40	ACA GTA CCC AAG CCC TGC TGT GCG CCC ACC CAG CTC AAC GCC ATC TCT	1315
	Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser	
	390 395 400	
45	GTC CTC TAC TTC GAC GAC AGC TCT AAT GTC ATC CTG AAG AAG TAC AGA	1363
	Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg	
	405 410 415 420	
50	AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCTTCC TGAGACCCTG	1413
	Asn Met Val Val Arg Ala Cys Gly Cys His	
	425 430	
50	ACCTTTGCGG GGCCACACCT TTCCAAATCT TCGATGTCTC ACCATCTAAG TCTCTCACTG	1473
	CCCACCTTGG CGAGGAGAAC AGACCAACCT CTCCTGAGCC TTCCCTCACC TCCCAACCGG	1533
	AAGCATGTAA GGGTTCCAGA AACCTGAGCG TGCAGCAGCT GATGAGCGCC CTTTCCTTCT	1593

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GGCACGTGAC GGACAAGATC CTACCAGCTA CCACAGCAAA CGCCTAAGAG CAGGAAAAAT 1653
 GTCTGCCAGG AAAGTGTCCA GTGTCCACAT GGCCCTGGC GCTCTGAGTC TTTGAGGAGT 1713
 5 AATCGCAAGC CTCGTTGAGC TGCAGCAGAA GGAAGGGCTT AGCCAGGGTG GGCCTGGCG 1773
 TCTGTGTTGA AGGGAAACCA AGCAGAAGCC ACTGTAATGA TATGTCACAA TAAACCCAT 1833
 GAATGAAAAA AAAAAAAAAA AAAAAAAAAA AAAAGAATTC 1873
 10

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 430 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala
 1 5 10 15
 25 Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser
 20 25 30
 Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser
 30 35 40 45
 Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu
 50 55 60
 35 Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
 65 70 75 80
 Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Ser Gly
 85 90 95
 40 Pro Asp Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr
 100 105 110
 Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp
 45 115 120 125
 Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu
 130 135 140

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	Phe	Phe	His	Pro	Arg	Tyr	His	His	Arg	Glu	Phe	Arg	Phe	Asp	Leu	Ser	
	145					150					155					160	
5	Lys	Ile	Pro	Glu	Gly	Glu	Arg	Val	Thr	Ala	Ala	Glu	Phe	Arg	Ile	Tyr	
					165					170					175		
	Lys	Asp	Tyr	Ile	Arg	Glu	Arg	Phe	Asp	Asn	Glu	Thr	Phe	Gln	Ile	Thr	
				180					185					190			
10	Val	Tyr	Gln	Val	Leu	Gln	Glu	His	Ser	Gly	Arg	Glu	Ser	Asp	Leu	Phe	
			195					200					205				
	Leu	Leu	Asp	Ser	Arg	Thr	Ile	Trp	Ala	Ser	Glu	Glu	Gly	Trp	Leu	Val	
15		210					215					220					
	Phe	Asp	Ile	Thr	Ala	Thr	Ser	Asn	His	Trp	Val	Val	Asn	Pro	Arg	His	
	225					230					235				240		
20	Asn	Leu	Gly	Leu	Gln	Leu	Ser	Val	Glu	Thr	Leu	Asp	Gly	Gln	Ser	Ile	
				245						250				255			
	Asn	Pro	Lys	Leu	Ala	Gly	Leu	Ile	Gly	Arg	His	Gly	Pro	Gln	Asn	Lys	
				260					265					270			
25	Gln	Pro	Phe	Met	Val	Ala	Phe	Phe	Lys	Ala	Thr	Glu	Val	His	Leu	Arg	
			275					280					285				
	Ser	Ile	Arg	Ser	Thr	Gly	Gly	Lys	Gln	Arg	Ser	Gln	Asn	Arg	Ser	Lys	
30		290					295					300					
	Thr	Pro	Lys	Asn	Gln	Glu	Ala	Leu	Arg	Met	Ala	Ser	Val	Ala	Glu	Asn	
	305					310					315				320		
35	Ser	Ser	Ser	Asp	Gln	Arg	Gln	Ala	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val	
				325					330					335			
	Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln	Asp	Trp	Ile	Ile	Ala	Pro	Glu	Gly	
				340					345					350			
40	Tyr	Ala	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala	Phe	Pro	Leu	Asn	Ser	
			355					360					365				
	Tyr	Met	Asn	Ala	Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu	Val	His	Phe	
45		370					375					380					
	Ile	Asn	Pro	Asp	Thr	Val	Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln	Leu	
	385					390					395				400		

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Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu
 405 410 415

5 Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
 420 425 430

(2) INFORMATION FOR SEQ ID NO:5:

10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1723 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens
 (F) TISSUE TYPE: HIPPOCAMPUS

20

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 490..1696
 (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
 25 /product= "hOP2-PP"
 /note= "hOP2 (cDNA)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

30 GGCGCCGGCA GAGCAGGAGT GGCTGGAGGA GCTGTGGTTG GAGCAGGAGG TGGCACGGCA 60
 GGGCTGGAGG GCTCCCTATG AGTGGCGGAG ACGGCCCAGG AGGCGCTGGA GCAACAGCTC 120
 35 CCACACCGCA CCAAGCGGTG GCTGCAGGAG CTCGCCCATC GCCCCTGCGC TGCTCGGACC 180
 GCGGCCACAG CCGGACTGGC GGGTACGGCG GCGACAGAGG CATTGGCCGA GAGTCCCAGT 240
 CCGCAGAGTA GCCCCGGCCT CGAGGCGGTG GCGTCCCGGT CCTCTCCGTC CAGGAGCCAG 300
 40 GACAGGTGTC GCGCGGCGGG GCTCCAGGA CCGCGCCTGA GGCCGGCTGC CCGCCCGTCC 360
 CGCCCCGCCC CGCCGCCCGC CGCCCGCCGA GCCCAGCCTC CTTGCCGTCG GGGCGTCCCC 420
 45 AGGCCCTGGG TCGGCCGCGG AGCCGATGCG CGCCCGCTGA GCGCCCCAGC TGAGCGCCCC 480
 CGGCCTGCC ATG ACC GCG CTC CCC GGC CCG CTC TGG CTC CTG GGC CTG 528
 Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu
 1 5 10

50

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	GCG	CTA	TGC	GCG	CTG	GGC	GGG	GGC	GGC	CCC	GGC	CTG	CGA	CCC	CCG	CCC	576
	Ala	Leu	Cys	Ala	Leu	Gly	Gly	Gly	Gly	Pro	Gly	Leu	Arg	Pro	Pro	Pro	
	15					20					25						
5	GGC	TGT	CCC	CAG	CGA	CGT	CTG	GGC	GCG	CGC	GAG	CGC	CGG	GAC	GTG	CAG	624
	Gly	Cys	Pro	Gln	Arg	Arg	Leu	Gly	Ala	Arg	Glu	Arg	Arg	Asp	Val	Gln	
	30					35					40					45	
10	CGC	GAG	ATC	CTG	GCG	GTG	CTC	GGG	CTG	CCT	GGG	CGG	CCC	CGG	CCC	CGC	672
	Arg	Glu	Ile	Leu	Ala	Val	Leu	Gly	Leu	Pro	Gly	Arg	Pro	Arg	Pro	Arg	
					50					55					60		
15	GCG	CCA	CCC	GCC	GCC	TCC	CGG	CTG	CCC	GCG	TCC	GCG	CCG	CTC	TTC	ATG	720
	Ala	Pro	Pro	Ala	Ala	Ser	Arg	Leu	Pro	Ala	Ser	Ala	Pro	Leu	Phe	Met	
				65					70					75			
20	CTG	GAC	CTG	TAC	CAC	GCC	ATG	GCC	GGC	GAC	GAC	GAC	GAG	GAC	GGC	GCG	768
	Leu	Asp	Leu	Tyr	His	Ala	Met	Ala	Gly	Asp	Asp	Asp	Glu	Asp	Gly	Ala	
			80					85					90				
25	CCC	GCG	GAG	CGG	CGC	CTG	GGC	CGC	GCC	GAC	CTG	GTC	ATG	AGC	TTC	GTT	816
	Pro	Ala	Glu	Arg	Arg	Leu	Gly	Arg	Ala	Asp	Leu	Val	Met	Ser	Phe	Val	
		95				100					105						
30	AAC	ATG	GTG	GAG	CGA	GAC	CGT	GCC	CTG	GGC	CAC	CAG	GAG	CCC	CAT	TGG	864
	Asn	Met	Val	Glu	Arg	Asp	Arg	Ala	Leu	Gly	His	Gln	Glu	Pro	His	Trp	
	110					115					120					125	
35	AAG	GAG	TTC	CGC	TTT	GAC	CTG	ACC	CAG	ATC	CCG	GCT	GGG	GAG	GCG	GTC	912
	Lys	Glu	Phe	Arg	Phe	Asp	Leu	Thr	Gln	Ile	Pro	Ala	Gly	Glu	Ala	Val	
					130					135					140		
40	ACA	GCT	GCG	GAG	TTC	CGG	ATT	TAC	AAG	GTG	CCC	AGC	ATC	CAC	CTG	CTC	960
	Thr	Ala	Ala	Glu	Phe	Arg	Ile	Tyr	Lys	Val	Pro	Ser	Ile	His	Leu	Leu	
				145					150					155			
45	AAC	AGG	ACC	CTC	CAC	GTC	AGC	ATG	TTC	CAG	GTG	GTC	CAG	GAG	CAG	TCC	1008
	Asn	Arg	Thr	Leu	His	Val	Ser	Met	Phe	Gln	Val	Val	Gln	Glu	Gln	Ser	
			160					165					170				
50	AAC	AGG	GAG	TCT	GAC	TTG	TTC	TTT	TTG	GAT	CTT	CAG	ACG	CTC	CGA	GCT	1056
	Asn	Arg	Glu	Ser	Asp	Leu	Phe	Phe	Leu	Asp	Leu	Gln	Thr	Leu	Arg	Ala	
		175				180						185					
55	GGA	GAC	GAG	GGC	TGG	CTG	GTG	CTG	GAT	GTC	ACA	GCA	GCC	AGT	GAC	TGC	1104
	Gly	Asp	Glu	Gly	Trp	Leu	Val	Leu	Asp	Val	Thr	Ala	Ala	Ser	Asp	Cys	
	190					195					200					205	

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	TGG	TTG	CTG	AAG	CGT	CAC	AAG	GAC	CTG	GGA	CTC	CGC	CTC	TAT	GTG	GAG	1152
	Trp	Leu	Leu	Lys	Arg	His	Lys	Asp	Leu	Gly	Leu	Arg	Leu	Tyr	Val	Glu	
					210					215					220		
5	ACT	GAG	GAC	GGG	CAC	AGC	GTG	GAT	CCT	GGC	CTG	GCC	GGC	CTG	CTG	GGT	1200
	Thr	Glu	Asp	Gly	His	Ser	Val	Asp	Pro	Gly	Leu	Ala	Gly	Leu	Leu	Gly	
				225					230					235			
10	CAA	CGG	GCC	CCA	CGC	TCC	CAA	CAG	CCT	TTC	GTG	GTC	ACT	TTC	TTC	AGG	1248
	Gln	Arg	Ala	Pro	Arg	Ser	Gln	Gln	Pro	Phe	Val	Val	Thr	Phe	Phe	Arg	
			240					245					250				
15	GCC	AGT	CCG	AGT	CCC	ATC	CGC	ACC	CCT	CGG	GCA	GTG	AGG	CCA	CTG	AGG	1296
	Ala	Ser	Pro	Ser	Pro	Ile	Arg	Thr	Pro	Arg	Ala	Val	Arg	Pro	Leu	Arg	
		255					260					265					
20	AGG	AGG	CAG	CCG	AAG	AAA	AGC	AAC	GAG	CTG	CCG	CAG	GCC	AAC	CGA	CTC	1344
	Arg	Arg	Gln	Pro	Lys	Lys	Ser	Asn	Glu	Leu	Pro	Gln	Ala	Asn	Arg	Leu	
	270					275				280						285	
25	CCA	GGG	ATC	TTT	GAT	GAC	GTC	CAC	GGC	TCC	CAC	GGC	CGG	CAG	GTC	TGC	1392
	Pro	Gly	Ile	Phe	Asp	Asp	Val	His	Gly	Ser	His	Gly	Arg	Gln	Val	Cys	
					290					295					300		
30	CGT	CGG	CAC	GAG	CTC	TAC	GTC	AGC	TTC	CAG	GAC	CTC	GGC	TGG	CTG	GAC	1440
	Arg	Arg	His	Glu	Leu	Tyr	Val	Ser	Phe	Gln	Asp	Leu	Gly	Trp	Leu	Asp	
				305					310					315			
35	TGG	GTC	ATC	GCT	CCC	CAA	GGC	TAC	TCG	GCC	TAT	TAC	TGT	GAG	GGG	GAG	1488
	Trp	Val	Ile	Ala	Pro	Gln	Gly	Tyr	Ser	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	
			320				325						330				
40	TGC	TCC	TTC	CCA	CTG	GAC	TCC	TGC	ATG	AAT	GCC	ACC	AAC	CAC	GCC	ATC	1536
	Cys	Ser	Phe	Pro	Leu	Asp	Ser	Cys	Met	Asn	Ala	Thr	Asn	His	Ala	Ile	
		335					340					345					
45	CTG	CAG	TCC	CTG	GTG	CAC	CTG	ATG	AAG	CCA	AAC	GCA	GTC	CCC	AAG	GCG	1584
	Leu	Gln	Ser	Leu	Val	His	Leu	Met	Lys	Pro	Asn	Ala	Val	Pro	Lys	Ala	
	350					355					360					365	
50	TGC	TGT	GCA	CCC	ACC	AAG	CTG	AGC	GCC	ACC	TCT	GTG	CTC	TAC	TAT	GAC	1632
	Cys	Cys	Ala	Pro	Thr	Lys	Leu	Ser	Ala	Thr	Ser	Val	Leu	Tyr	Tyr	Asp	
					370					375					380		
55	AGC	AGC	AAC	AAC	GTC	ATC	CTG	CGC	AAA	GCC	CGC	AAC	ATG	GTG	GTC	AAG	1680
	Ser	Ser	Asn	Asn	Val	Ile	Leu	Arg	Lys	Ala	Arg	Asn	Met	Val	Val	Lys	
				385					390					395			
60	GCC	TGC	GGC	TGC	CAC	T	GAGTCAGCCC	GCCCAGCCCT	ACTGCAG								1723
	Ala	Cys	Gly</														

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(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 402 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys
1 5 10 15

15 Ala Leu Gly Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro Gly Cys Pro
20 25 30

Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln Arg Glu Ile
35 40 45

20 Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Pro Pro
50 55 60

25 Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu
65 70 75 80

Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala Pro Ala Glu
85 90 95

30 Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val Asn Met Val
100 105 110

Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp Lys Glu Phe
115 120 125

35 Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala
130 135 140

40 Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu Asn Arg Thr
145 150 155 160

Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser Asn Arg Glu
165 170 175

45 Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala Gly Asp Glu
180 185 190

Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys Trp Leu Leu
195 200 205

50

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Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Glu Asp
 210 215 220
 Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly Gln Arg Ala
 5 225 230 235 240
 Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg Ala Ser Pro
 245 250 255
 10 Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg Arg Arg Gln
 260 265 270
 Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu Pro Gly Ile
 275 280 285
 15 Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys Arg Arg His
 290 295 300
 Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp Trp Val Ile
 20 305 310 315 320
 Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ser Phe
 325 330 335
 25 Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser
 340 345 350
 Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala Cys Cys Ala
 355 360 365
 30 Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn
 370 375 380
 Asn Val Ile Leu Arg Lys Ala Arg Asn Met Val Val Lys Ala Cys Gly
 35 385 390 395 400
 Cys His

40 (2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1926 base pairs
 (B) TYPE: nucleic acid
 45 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: MURIDAE
 50 (F) TISSUE TYPE: EMBRYO

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(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 93..1289

(D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"

5 /product= "mOP2-PP"

/note= "mOP2 cDNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

10 GCCAGGCACA GGTGCGCCGT CTGGTCCTCC CCGTCTGGCG TCAGCCGAGC CCGACCAGCT 60

ACCAGTGGAT GCGCGCCGGC TGAAAGTCCG AG ATG GCT ATG CGT CCC GGG CCA 113
Met Ala Met Arg Pro Gly Pro

15 1 5

CTC TGG CTA TTG GGC CTT GCT CTG TGC GCG CTG GGA GGC GGC CAC GGT 161
Leu Trp Leu Leu Gly Leu Ala Leu Cys Ala Leu Gly Gly Gly His Gly

20 10 15 20

CCG CGT CCC CCG CAC ACC TGT CCC CAG CGT CGC CTG GGA GCG CGC GAG 209
Pro Arg Pro Pro His Thr Cys Pro Gln Arg Arg Leu Gly Ala Arg Glu

25 25 30 35

CGC CGC GAC ATG CAG CGT GAA ATC CTG GCG GTG CTC GGG CTA CCG GGA 257
Arg Arg Asp Met Gln Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly

30 40 45 50 55

CGC CCC CGA CCC CGT GCA CAA CCC GCC GCT GCC CGG CAG CCA GCG TCC 305
Arg Pro Arg Pro Arg Ala Gln Pro Ala Ala Ala Arg Gln Pro Ala Ser

35 60 65 70

GCG CCC CTC TTC ATG TTG GAC CTA TAC CAC GCC ATG ACC GAT GAC GAC 353
Ala Pro Leu Phe Met Leu Asp Leu Tyr His Ala Met Thr Asp Asp Asp

40 75 80 85

GAC GGC GGG CCA CCA CAG GCT CAC TTA GGC CGT GCC GAC CTG GTC ATG 401
Asp Gly Gly Pro Pro Gln Ala His Leu Gly Arg Ala Asp Leu Val Met

45 90 95 100

AGC TTC GTC AAC ATG GTG GAA CGC GAC CGT ACC CTG GGC TAC CAG GAG 449
Ser Phe Val Asn Met Val Glu Arg Asp Arg Thr Leu Gly Tyr Gln Glu

105 110 115

CCA CAC TGG AAG GAA TTC CAC TTT GAC CTA ACC CAG ATC CCT GCT GGG 497
Pro His Trp Lys Glu Phe His Phe Asp Leu Thr Gln Ile Pro Ala Gly

120 125 130 135

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	GAG	GCT	GTC	ACA	GCT	GCT	GAG	TTC	CGG	ATC	TAC	AAA	GAA	CCC	AGC	ACC	545
	Glu	Ala	Val	Thr	Ala	Ala	Glu	Phe	Arg	Ile	Tyr	Lys	Glu	Pro	Ser	Thr	
					140					145						150	
5	CAC	CCG	CTC	AAC	ACA	ACC	CTC	CAC	ATC	AGC	ATG	TTC	GAA	GTG	GTC	CAA	593
	His	Pro	Leu	Asn	Thr	Thr	Leu	His	Ile	Ser	Met	Phe	Glu	Val	Val	Gln	
				155					160					165			
	GAG	CAC	TCC	AAC	AGG	GAG	TCT	GAC	TTG	TTC	TTT	TTG	GAT	CTT	CAG	ACG	641
10	Glu	His	Ser	Asn	Arg	Glu	Ser	Asp	Leu	Phe	Phe	Leu	Asp	Leu	Gln	Thr	
			170					175					180				
	CTC	CGA	TCT	GGG	GAC	GAG	GGC	TGG	CTG	GTG	CTG	GAC	ATC	ACA	GCA	GCC	689
15	Leu	Arg	Ser	Gly	Asp	Glu	Gly	Trp	Leu	Val	Leu	Asp	Ile	Thr	Ala	Ala	
		185					190					195					
	AGT	GAC	CGA	TGG	CTG	CTG	AAC	CAT	CAC	AAG	GAC	CTG	GGA	CTC	CGC	CTC	737
	Ser	Asp	Arg	Trp	Leu	Leu	Asn	His	His	Lys	Asp	Leu	Gly	Leu	Arg	Leu	
	200					205					210					215	
20	TAT	GTG	GAA	ACC	GCG	GAT	GGG	CAC	AGC	ATG	GAT	CCT	GGC	CTG	GCT	GGT	785
	Tyr	Val	Glu	Thr	Ala	Asp	Gly	His	Ser	Met	Asp	Pro	Gly	Leu	Ala	Gly	
					220					225					230		
25	CTG	CTT	GGA	CGA	CAA	GCA	CCA	CGC	TCC	AGA	CAG	CCT	TTC	ATG	GTA	ACC	833
	Leu	Leu	Gly	Arg	Gln	Ala	Pro	Arg	Ser	Arg	Gln	Pro	Phe	Met	Val	Thr	
				235					240					245			
30	TTC	TTC	AGG	GCC	AGC	CAG	AGT	CCT	GTG	CGG	GCC	CCT	CGG	GCA	GCG	AGA	881
	Phe	Phe	Arg	Ala	Ser	Gln	Ser	Pro	Val	Arg	Ala	Pro	Arg	Ala	Ala	Arg	
			250					255					260				
	CCA	CTG	AAG	AGG	AGG	CAG	CCA	AAG	AAA	ACG	AAC	GAG	CTT	CCG	CAC	CCC	929
35	Pro	Leu	Lys	Arg	Arg	Gln	Pro	Lys	Lys	Thr	Asn	Glu	Leu	Pro	His	Pro	
		265					270					275					
	AAC	AAA	CTC	CCA	GGG	ATC	TTT	GAT	GAT	GGC	CAC	GGT	TCC	CGC	GGC	AGA	977
	Asn	Lys	Leu	Pro	Gly	Ile	Phe	Asp	Asp	Gly	His	Gly	Ser	Arg	Gly	Arg	
	280					285					290					295	
40	GAG	GTT	TGC	CGC	AGG	CAT	GAG	CTC	TAC	GTC	AGC	TTC	CGT	GAC	CTT	GGC	1025
	Glu	Val	Cys	Arg	Arg	His	Glu	Leu	Tyr	Val	Ser	Phe	Arg	Asp	Leu	Gly	
					300					305					310		
45	TGG	CTG	GAC	TGG	GTC	ATC	GCC	CCC	CAG	GGC	TAC	TCT	GCC	TAT	TAC	TGT	1073
	Trp	Leu	Asp	Trp	Val	Ile	Ala	Pro	Gln	Gly	Tyr	Ser	Ala	Tyr	Tyr	Cys	
				315					320					325			

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	GAG GGG GAG TGT GCT TTC CCA CTG GAC TCC TGT ATG AAC GCC ACC AAC	1121
	Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn	
	330 335 340	
5	CAT GCC ATC TTG CAG TCT CTG GTG CAC CTG ATG AAG CCA GAT GTT GTC	1169
	His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro Asp Val Val	
	345 350 355	
10	CCC AAG GCA TGC TGT GCA CCC ACC AAA CTG AGT GCC ACC TCT GTG CTG	1217
	Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu	
	360 365 370 375	
15	TAC TAT GAC AGC AGC AAC AAT GTC ATC CTG CGT AAA CAC CGT AAC ATG	1265
	Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His Arg Asn Met	
	380 385 390	
	GTG GTC AAG GCC TGT GGC TGC CAC TGAGGCCCCG CCCAGCATCC TGCTTCTACT	1319
	Val Val Lys Ala Cys Gly Cys His	
	395	
20	ACCTTACCAT CTGGCCGGGC CCCTCTCCAG AGGCAGAAAC CCTTCTATGT TATCATAGCT	1379
	CAGACAGGGG CAATGGGAGG CCCTTCACTT CCCCTGGCCA CTTCTGTGCTA AAATTCTGGT	1439
25	CTTTCCCAGT TCCTCTGTCC TTCATGGGGT TTCGGGGCTA TCACCCCGCC CTCTCCATCC	1499
	TCCTACCCCA AGCATAGACT GAATGCACAC AGCATCCCAG AGCTATGCTA ACTGAGAGGT	1559
	CTGGGGTCAG CACTGAAGGC CCACATGAGG AAGACTGATC CTTGGCCATC CTCAGCCCAC	1619
30	AATGGCAAAT TCTGGATGGT CTAAGAAGGC CCTGGAATTC TAAACTAGAT GATCTGGGCT	1679
	CTCTGCACCA TTCATTGTGG CAGTTGGGAC ATTTTITAGGT ATAACAGACA CATACTTIA	1739
35	GATCAATGCA TCGCTGTACT CCTTGAAATC AGAGCTAGCT TGTTAGAAAA AGAATCAGAG	1799
	CCAGGTATAG CGGTGCATGT CATTAATCCC AGCGCTAAAG AGACAGAGAC AGGAGAATCT	1859
	CTGTGAGTTC AAGGCCACAT AGAAAGAGCC TGTCTCGGGA GCAGGAAAAA AAAAAAAAAAC	1919
40	GGAATTC	1926

(2) INFORMATION FOR SEQ ID NO:8:

45

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 399 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

50

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(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

5	Met	Ala	Met	Arg	Pro	Gly	Pro	Leu	Trp	Leu	Leu	Gly	Leu	Ala	Leu	Cys
	1				5					10					15	
	Ala	Leu	Gly	Gly	Gly	His	Gly	Pro	Arg	Pro	Pro	His	Thr	Cys	Pro	Gln
			20						25					30		
10	Arg	Arg	Leu	Gly	Ala	Arg	Glu	Arg	Arg	Asp	Met	Gln	Arg	Glu	Ile	Leu
			35					40					45			
	Ala	Val	Leu	Gly	Leu	Pro	Gly	Arg	Pro	Arg	Pro	Arg	Ala	Gln	Pro	Ala
15		50					55					60				
	Ala	Ala	Arg	Gln	Pro	Ala	Ser	Ala	Pro	Leu	Phe	Met	Leu	Asp	Leu	Tyr
	65					70					75					80
20	His	Ala	Met	Thr	Asp	Asp	Asp	Asp	Gly	Gly	Pro	Pro	Gln	Ala	His	Leu
				85						90					95	
	Gly	Arg	Ala	Asp	Leu	Val	Met	Ser	Phe	Val	Asn	Met	Val	Glu	Arg	Asp
			100						105					110		
25	Arg	Thr	Leu	Gly	Tyr	Gln	Glu	Pro	His	Trp	Lys	Glu	Phe	His	Phe	Asp
		115						120					125			
	Leu	Thr	Gln	Ile	Pro	Ala	Gly	Glu	Ala	Val	Thr	Ala	Ala	Glu	Phe	Arg
30		130					135						140			
	Ile	Tyr	Lys	Glu	Pro	Ser	Thr	His	Pro	Leu	Asn	Thr	Thr	Leu	His	Ile
	145					150					155					160
35	Ser	Met	Phe	Glu	Val	Val	Gln	Glu	His	Ser	Asn	Arg	Glu	Ser	Asp	Leu
				165						170					175	
	Phe	Phe	Leu	Asp	Leu	Gln	Thr	Leu	Arg	Ser	Gly	Asp	Glu	Gly	Trp	Leu
			180						185					190		
40	Val	Leu	Asp	Ile	Thr	Ala	Ala	Ser	Asp	Arg	Trp	Leu	Leu	Asn	His	His
		195						200					205			
	Lys	Asp	Leu	Gly	Leu	Arg	Leu	Tyr	Val	Glu	Thr	Ala	Asp	Gly	His	Ser
45		210					215					220				
	Met	Asp	Pro	Gly	Leu	Ala	Gly	Leu	Leu	Gly	Arg	Gln	Ala	Pro	Arg	Ser
	225					230					235					240

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Arg Gln Pro Phe Met Val Thr Phe Phe Arg Ala Ser Gln Ser Pro Val
 245 250 255
 5 Arg Ala Pro Arg Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys
 260 265 270
 Thr Asn Glu Leu Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp
 275 280 285
 10 Gly His Gly Ser Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr
 290 295 300
 Val Ser Phe Arg Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln
 15 305 310 315 320
 Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp
 325 330 335
 20 Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His
 340 345 350
 Leu Met Lys Pro Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys
 355 360 365
 25 Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile
 370 375 380
 Leu Arg Lys His Arg Asn Met Val Val Lys Ala Cys Gly Cys His
 30 385 390 395

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH: 399 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

40

(ix) FEATURE:

- 45 (A) NAME/KEY: Protein
 (B) LOCATION: 1..399
 (D) OTHER INFORMATION: /note= "PRE-PRO-OP3 (MOUSE)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

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	Met	Ala	Ala	Arg	Pro	Gly	Leu	Leu	Trp	Leu	Leu	Gly	Leu	Ala	Leu	Cys
	1				5					10					15	
5	Val	Leu	Gly	Gly	Gly	His	Leu	Ser	His	Pro	Pro	His	Val	Phe	Pro	Gln
				20					25					30		
	Arg	Arg	Leu	Gly	Val	Arg	Glu	Pro	Arg	Asp	Met	Gln	Arg	Glu	Ile	Arg
			35					40					45			
10	Glu	Val	Leu	Gly	Leu	Ala	Gly	Arg	Pro	Arg	Ser	Arg	Ala	Pro	Val	Gly
	50						55					60				
	Ala	Ala	Gln	Gln	Pro	Ala	Ser	Ala	Pro	Leu	Phe	Met	Leu	Asp	Leu	Tyr
15	65					70					75					80
	Arg	Ala	Met	Thr	Asp	Asp	Ser	Gly	Gly	Gly	Thr	Pro	Gln	Pro	His	Leu
					85					90					95	
20	Asp	Arg	Ala	Asp	Leu	Ile	Met	Ser	Phe	Val	Asn	Ile	Val	Glu	Arg	Asp
				100					105					110		
	Arg	Thr	Leu	Gly	Tyr	Gln	Glu	Pro	His	Trp	Lys	Glu	Phe	His	Phe	Asp
			115					120					125			
25	Leu	Thr	Gln	Ile	Pro	Ala	Gly	Glu	Ala	Val	Thr	Ala	Ala	Glu	Phe	Arg
							135						140			
	Ile	Tyr	Lys	Glu	Pro	Ser	Thr	His	Pro	Leu	Asn	Thr	Thr	Leu	His	Ile
30	145					150					155					160
	Ser	Met	Phe	Glu	Val	Val	Gln	Glu	His	Ser	Asn	Arg	Glu	Ser	Asp	Leu
					165					170					175	
35	Phe	Phe	Leu	Asp	Leu	Gln	Thr	Leu	Arg	Ser	Gly	Asp	Glu	Gly	Trp	Leu
				180					185					190		
	Val	Leu	Asp	Ile	Thr	Ala	Ala	Ser	Asp	Arg	Trp	Leu	Leu	Asn	His	His
			195					200					205			
40	Lys	Asp	Leu	Gly	Leu	Arg	Leu	Tyr	Val	Glu	Thr	Glu	Asp	Gly	His	Ser
	210						215					220				
	Ile	Asp	Pro	Gly	Leu	Ala	Gly	Leu	Leu	Gly	Arg	Gln	Ala	Pro	Arg	Ser
45	225					230					235					240
	Arg	Gln	Pro	Phe	Met	Val	Gly	Phe	Phe	Arg	Ala	Asn	Gln	Ser	Pro	Val
					245					250					255	

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	Arg	Ala	Pro	Arg	Thr	Ala	Arg	Pro	Leu	Lys	Lys	Lys	Gln	Leu	Asn	Gln
				260					265					270		
5	Ile	Asn	Gln	Leu	Pro	His	Ser	Asn	Lys	His	Leu	Gly	Ile	Leu	Asp	Asp
			275					280					285			
	Gly	His	Gly	Ser	His	Gly	Arg	Glu	Val	Cys	Arg	Arg	His	Glu	Leu	Tyr
		290					295					300				
10	Val	Ser	Phe	Arg	Asp	Leu	Gly	Trp	Leu	Asp	Ser	Val	Ile	Ala	Pro	Gln
	305					310					315					320
	Gly	Tyr	Ser	Ala	Tyr	Tyr	Cys	Ala	Gly	Glu	Cys	Ile	Tyr	Pro	Leu	Asn
					325					330					335	
15	Ser	Cys	Met	Asn	Ser	Thr	Asn	His	Ala	Thr	Met	Gln	Ala	Leu	Val	His
				340					345					350		
	Leu	Met	Lys	Pro	Asp	Ile	Ile	Pro	Lys	Val	Cys	Cys	Val	Pro	Thr	Glu
20			355					360					365			
	Leu	Ser	Ala	Ile	Ser	Leu	Leu	Tyr	Tyr	Asp	Arg	Asn	Asn	Asn	Val	Ile
		370					375					380				
25	Leu	Arg	Arg	Glu	Arg	Asn	Met	Val	Val	Gln	Ala	Cys	Gly	Cys	His	
	385					390					395					

(2) INFORMATION FOR SEQ ID NO:10:

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30      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 396 amino acids
          (B) TYPE: amino acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear
35
      (ii) MOLECULE TYPE: protein

40      (ix) FEATURE:
          (A) NAME/KEY: Protein
          (B) LOCATION: 1..396
          (D) OTHER INFORMATION: /note= "PRE-PRO-BMP2 (HUMAN)"

45      (x) PUBLICATION INFORMATION:
          (A) AUTHORS: WOZNEY,
          (C) JOURNAL: SCIENCE
          (D) VOLUME: 242
          (F) PAGES: 1528-1534
          (G) DATE: 1988
50

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

5	Met	Val	Ala	Gly	Thr	Arg	Cys	Leu	Leu	Ala	Leu	Leu	Leu	Pro	Gln	Val	1	5	10	15	
	Leu	Leu	Gly	Gly	Ala	Ala	Gly	Leu	Val	Pro	Glu	Leu	Gly	Arg	Arg	Lys	20	25	30		
10	Phe	Ala	Ala	Ala	Ser	Ser	Gly	Arg	Pro	Ser	Ser	Gln	Pro	Ser	Asp	Glu	35	40	45		
	Val	Leu	Ser	Glu	Phe	Glu	Leu	Arg	Leu	Leu	Ser	Met	Phe	Gly	Leu	Lys	50	55	60		
15	Gln	Arg	Pro	Thr	Pro	Ser	Arg	Asp	Ala	Val	Val	Pro	Pro	Tyr	Met	Leu	65	70	75	80	
	Asp	Leu	Tyr	Arg	Arg	His	Ser	Gly	Gln	Pro	Gly	Ser	Pro	Ala	Pro	Asp	85	90	95		
20	His	Arg	Leu	Glu	Arg	Ala	Ala	Ser	Arg	Ala	Asn	Thr	Val	Arg	Ser	Phe	100	105	110		
	His	His	Glu	Glu	Ser	Leu	Glu	Glu	Leu	Pro	Glu	Thr	Ser	Gly	Lys	Thr	115	120	125		
25	Thr	Arg	Arg	Phe	Phe	Phe	Asn	Leu	Ser	Ser	Ile	Pro	Thr	Glu	Glu	Phe	130	135	140		
30	Ile	Thr	Ser	Ala	Glu	Leu	Gln	Val	Phe	Arg	Glu	Gln	Met	Gln	Asp	Ala	145	150	155	160	
	Leu	Gly	Asn	Asn	Ser	Ser	Phe	His	His	Arg	Ile	Asn	Ile	Tyr	Glu	Ile	165	170	175		
35	Ile	Lys	Pro	Ala	Thr	Ala	Asn	Ser	Lys	Phe	Pro	Val	Thr	Arg	Leu	Leu	180	185	190		
40	Asp	Thr	Arg	Leu	Val	Asn	Gln	Asn	Ala	Ser	Arg	Trp	Glu	Ser	Phe	Asp	195	200	205		
	Val	Thr	Pro	Ala	Val	Met	Arg	Trp	Thr	Ala	Gln	Gly	His	Ala	Asn	His	210	215	220		
45	Gly	Phe	Val	Val	Glu	Val	Ala	His	Leu	Glu	Glu	Lys	Gln	Gly	Val	Ser	Lys	225	230	235	240
	Arg	His	Val	Arg	Ile	Ser	Arg	Ser	Leu	His	Gln	Asp	Glu	His	Ser	Trp	245	250	255		
50																					

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Ser Gln Ile Arg Pro Leu Leu Val Thr Phe Gly His Asp Gly Lys Gly
 260 265 270
 5 His Pro Leu His Lys Arg Glu Lys Arg Gln Ala Lys His Lys Gln Arg
 275 280 285
 Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp Phe
 290 295 300 305
 10 Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr His
 310 315 320
 Ala Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His Leu
 325 330 335
 15 Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn
 340 345 350
 Ser Lys Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile
 355 360 365 370
 20 Ser Met Leu Tyr Leu Asp Glu Asn Glu Lys Val Val Leu Lys Asn Tyr
 375 380 385
 25 Gln Asp Met Val Val Glu Gly Cys Gly Cys Arg
 390 395

(2) INFORMATION FOR SEQ ID NO:11:

- 30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 408 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 35 (ii) MOLECULE TYPE: protein
 (ix) FEATURE:
 40 (A) NAME/KEY: Protein
 (B) LOCATION: 1..408
 (D) OTHER INFORMATION: /note= "PRE-PRO-BMP4 (HUMAN)"
 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

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	Met	Ile	Pro	Gly	Asn	Arg	Met	Leu	Met	Val	Val	Leu	Leu	Cys	Gln	Val	
	1				5					10					15		
5	Leu	Leu	Gly	Gly	Ala	Ser	His	Ala	Ser	Leu	Ile	Pro	Glu	Thr	Gly	Lys	
				20					25					30			
	Lys	Lys	Val	Ala	Glu	Ile	Gln	Gly	His	Ala	Gly	Gly	Arg	Arg	Ser	Gly	
			35					40					45				
10	Gln	Ser	His	Glu	Leu	Leu	Arg	Asp	Phe	Glu	Ala	Thr	Leu	Leu	Gln	Met	
		50					55					60					
	Phe	Gly	Leu	Arg	Arg	Arg	Pro	Gln	Pro	Ser	Lys	Ser	Ala	Val	Ile	Pro	
	65					70					75					80	
15	Asp	Tyr	Met	Arg	Asp	Leu	Tyr	Arg	Leu	Gln	Ser	Gly	Glu	Glu	Glu	Glu	
					85					90					95		
	Glu	Gln	Ile	His	Ser	Thr	Gly	Leu	Glu	Tyr	Pro	Glu	Arg	Pro	Ala	Ser	
20				100					105					110			
	Arg	Ala	Asn	Thr	Val	Arg	Ser	Phe	His	His	Glu	Glu	His	Leu	Glu	Asn	
			115					120					125				
25	Ile	Pro	Gly	Thr	Ser	Glu	Asn	Ser	Ala	Phe	Arg	Phe	Leu	Phe	Asn	Leu	
		130					135					140					
	Ser	Ser	Ile	Pro	Glu	Asn	Glu	Val	Ile	Ser	Ser	Ala	Glu	Leu	Arg	Leu	
	145					150					155					160	
30	Phe	Arg	Glu	Gln	Val	Asp	Gln	Gly	Pro	Asp	Trp	Glu	Arg	Gly	Phe	His	
					165					170					175		
	Arg	Ile	Asn	Ile	Tyr	Glu	Val	Met	Lys	Pro	Pro	Ala	Glu	Val	Val	Pro	
35				180					185					190			
	Gly	His	Leu	Ile	Thr	Arg	Leu	Leu	Asp	Thr	Arg	Leu	Val	His	His	Asn	
			195					200					205				
40	Val	Thr	Arg	Trp	Glu	Thr	Phe	Asp	Val	Ser	Pro	Ala	Val	Leu	Arg	Trp	
		210					215					220					
	Thr	Arg	Glu	Lys	Gln	Pro	Asn	Tyr	Gly	Leu	Ala	Ile	Glu	Val	Thr	His	
	225					230					235					240	
45	Leu	His	Gln	Thr	Arg	Thr	His	Gln	Gly	Gln	His	Val	Arg	Ile	Ser	Arg	
					245					250					255		

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Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu
 260 265 270
 5 Val Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg
 275 280 285
 Arg Ala Lys Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys
 290 295 300
 10 Asn Lys Asn Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Phe Asp
 305 310 315 320
 Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe
 325 330 335
 15 Tyr Cys His Gly Asp Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser
 340 345 350
 Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser
 355 360 365
 20 Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met
 370 375 380
 25 Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu
 385 390 395
 Met Val Val Glu Gly Cys Gly Cys Arg
 400 405

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH: 588 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- 40 (A) NAME/KEY: Protein
 (B) LOCATION: 1..588
 45 (D) OTHER INFORMATION: /note= "PRE-PRO-DPP"

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(x) PUBLICATION INFORMATION:

(A) AUTHORS: PADGETT,

(C) JOURNAL: NATURE

(D) VOLUME: 325

5 (F) PAGES: 81-84

(G) DATE: 1987

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

10	Met	Arg	Ala	Trp	Leu	Leu	Leu	Leu	Ala	Val	Leu	Ala	Thr	Phe	Gln	Thr
	1				5					10					15	
	Ile	Val	Arg	Val	Ala	Ser	Thr	Glu	Asp	Ile	Ser	Gln	Arg	Phe	Ile	Ala
				20					25					30		
15	Ala	Ile	Ala	Pro	Val	Ala	Ala	His	Ile	Pro	Leu	Ala	Ser	Ala	Ser	Gly
			35					40					45			
	Ser	Gly	Ser	Gly	Arg	Ser	Gly	Ser	Arg	Ser	Val	Gly	Ala	Ser	Thr	Ser
20		50					55					60				
	Thr	Ala	Leu	Ala	Lys	Ala	Phe	Asn	Pro	Phe	Ser	Glu	Pro	Ala	Ser	Phe
	65					70					75					80
25	Ser	Asp	Ser	Asp	Lys	Ser	His	Arg	Ser	Lys	Thr	Asn	Lys	Lys	Pro	Ser
					85					90					95	
	Lys	Ser	Asp	Ala	Asn	Arg	Gln	Phe	Asn	Glu	Val	His	Lys	Pro	Arg	Thr
				100					105					110		
30	Asp	Gln	Leu	Glu	Asn	Ser	Lys	Asn	Lys	Ser	Lys	Gln	Leu	Val	Asn	Lys
			115					120					125			Pro
	Asn	His	Asn	Lys	Met	Ala	Val	Lys	Glu	Gln	Arg	Ser	His	His	Lys	Lys
35	130					135					140					145
	Ser	His	His	His	Arg	Ser	His	Gln	Pro	Lys	Gln	Ala	Ser	Ala	Ser	Thr
					150					155					160	
40	Glu	Ser	His	Gln	Ser	Ser	Ser	Ile	Glu	Ser	Ile	Phe	Val	Glu	Glu	Pro
				165					170					175		
	Thr	Leu	Val	Leu	Asp	Arg	Glu	Val	Ala	Ser	Ile	Asn	Val	Pro	Ala	Ser
				180				185					190			
45	Ala	Lys	Ala	Ile	Ile	Ala	Glu	Gln	Gly	Pro	Ser	Thr	Tyr	Ser	Lys	Glu
		195					200					205				
	Ala	Leu	Ile	Lys	Asp	Lys	Leu	Lys	Pro	Asp	Pro	Ser	Thr	Leu	Val	Glu
50	210					215					220					225

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	Ile	Glu	Lys	Ser	Leu	Leu	Ser	Leu	Phe	Asn	Met	Lys	Arg	Pro	Pro	Lys	
					230					235					240		
5	Ile	Asp	Arg	Ser	Lys	Ile	Ile	Ile	Pro	Glu	Pro	Met	Lys	Lys	Leu	Tyr	
				245					250					255			
	Ala	Glu	Ile	Met	Gly	His	Glu	Leu	Asp	Ser	Val	Asn	Ile	Pro	Lys	Pro	
				260				265					270				
10	Gly	Leu	Leu	Thr	Lys	Ser	Ala	Asn	Thr	Val	Arg	Ser	Phe	Thr	His	Lys	
		275					280					285					
	Asp	Ser	Lys	Ile	Asp	Asp	Arg	Phe	Pro	His	His	His	Arg	Phe	Arg	Leu	
	290					295					300					305	
15	His	Phe	Asp	Val	Lys	Ser	Ile	Pro	Ala	Asp	Glu	Lys	Leu	Lys	Ala	Ala	
					310					315					320		
	Glu	Leu	Gln	Leu	Thr	Arg	Asp	Ala	Leu	Ser	Gln	Gln	Val	Val	Ala	Ser	
20				325					330					335			
	Arg	Ser	Ser	Ala	Asn	Arg	Thr	Arg	Tyr	Gln	Val	Leu	Val	Tyr	Asp	Ile	
			340					345					350				
25	Thr	Arg	Val	Gly	Val	Arg	Gly	Gln	Arg	Glu	Pro	Ser	Tyr	Leu	Leu	Leu	
		355					360					365					
	Asp	Thr	Lys	Thr	Val	Arg	Leu	Asn	Ser	Thr	Asp	Thr	Val	Ser	Leu	Asp	
	370					375					380					385	
30	Val	Gln	Pro	Ala	Val	Asp	Arg	Trp	Leu	Ala	Ser	Pro	Gln	Arg	Asn	Tyr	
					390					395					400		
	Gly	Leu	Leu	Val	Glu	Val	Arg	Thr	Val	Arg	Ser	Leu	Lys	Pro	Ala	Pro	
35				405					410					415			
	His	His	His	Val	Arg	Leu	Arg	Arg	Ser	Ala	Asp	Glu	Ala	His	Glu	Arg	
			420					425					430				
40	Trp	Gln	His	Lys	Gln	Pro	Leu	Leu	Phe	Thr	Tyr	Thr	Asp	Asp	Gly	Arg	
		435					440					445					
	His	Lys	Ala	Arg	Ser	Ile	Arg	Asp	Val	Ser	Gly	Gly	Glu	Gly	Gly	Gly	
	450					455					460					465	
45	Lys	Gly	Gly	Arg	Asn	Lys	Arg	His	Ala	Arg	Arg	Pro	Thr	Arg	Arg	Lys	
					470					475					480		
	Asn	His	Asp	Asp	Thr	Cys	Arg	Arg	His	Ser	Leu	Tyr	Val	Asp	Phe	Ser	
50				485					490					495			

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Asp Val Gly Trp Asp Asp Trp Ile Val Ala Pro Leu Gly Tyr Asp Ala
 500 505 510
 5 Tyr Tyr Cys His Gly Lys Cys Pro Phe Pro Leu Ala Asp His Phe Asn
 515 520 525
 Ser Thr Asn His Ala Val Val Gln Thr Leu Val Asn Asn Met Asn Pro
 530 535 540 545
 10 Gly Lys Val Pro Lys Ala Cys Cys Val Pro Thr Gln Leu Asp Ser Val
 550 555
 Ala Met Leu Tyr Leu Asn Asp Gln Ser Thr Val Val Leu Lys Asn Tyr
 565 570 575
 15 Gln Glu Met Thr Val Val Gly Cys Gly Cys Arg
 580 585

(2) INFORMATION FOR SEQ ID NO:13:

20

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 359 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

25

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

30

(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..359

(D) OTHER INFORMATION: /note= "PRE-PRO-VG1"

35

(x) PUBLICATION INFORMATION:

(A) AUTHORS: WEEKS,

(C) JOURNAL: CELL

(D) VOLUME: 51

(F) PAGES: 861-867

40

(G) DATE: 1987

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met Val Trp Leu Arg Leu Trp Ala Phe Leu His Ile Leu Ala Ile Val
 1 5 10 15
 Thr Leu Asp Pro Glu Leu Lys Arg Arg Glu Glu Leu Phe Leu Arg Ser
 20 25 30
 50 Leu Gly Phe Ser Ser Lys Pro Asn Pro Val Ser Pro Pro Pro Val Pro
 35 40 45

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	Ser	Ile	Leu	Trp	Arg	Ile	Phe	Asn	Gln	Arg	Met	Gly	Ser	Ser	Ile	Gln	
	50						55					60					
5	Lys	Lys	Lys	Pro	Asp	Leu	Cys	Phe	Val	Glu	Glu	Phe	Asn	Val	Pro	Gly	
	65					70				75						80	
	Ser	Val	Ile	Arg	Val	Phe	Pro	Asp	Gln	Gly	Arg	Phe	Ile	Ile	Pro	Tyr	
					85					90					95		
10	Ser	Asp	Asp	Ile	His	Pro	Thr	Gln	Cys	Leu	Glu	Lys	Arg	Leu	Phe	Phe	
				100					105						110		
	Asn	Ile	Ser	Ala	Ile	Glu	Lys	Glu	Glu	Arg	Val	Thr	Met	Gly	Ser	Gly	
15			115					120					125				
	Ile	Glu	Val	Glu	Pro	Glu	His	Leu	Leu	Arg	Lys	Gly	Ile	Asp	Leu	Arg	
	130					135						140					
20	Leu	Tyr	Arg	Thr	Leu	Gln	Ile	Thr	Leu	Lys	Gly	Met					
	145					150					155						
	Gly	Arg	Ser	Lys	Thr	Ser	Arg	Lys	Leu	Leu	Val	Ala	Gln	Thr	Phe	Arg	
				160					165						170		
25	Leu	Leu	His	Lys	Ser	Leu	Phe	Phe	Asn	Leu	Thr	Glu	Ile	Cys	Gln	Ser	
			180					185					190				
	Trp	Gln	Asp	Pro	Leu	Lys	Asn	Leu	Gly	Leu	Val	Leu	Glu	Ile	Phe	Pro	
30		195					200					205					
	Lys	Lys	Glu	Ser	Ser	Trp	Met	Ser	Thr	Ala	Asn	Asp	Glu	Cys	Lys	Asp	Ile
	210					215					220					225	
35	Gln	Thr	Phe	Leu	Tyr	Thr	Ser	Leu	Leu	Thr	Val	Thr	Leu	Asn	Pro	Leu	
				230					235						240		
	Arg	Cys	Lys	Arg	Pro	Arg	Arg	Lys	Arg	Ser	Tyr	Ser	Lys	Leu	Pro	Phe	
			245					250					255				
40	Thr	Ala	Ser	Asn	Ile	Cys	Lys	Lys	Arg	His	Leu	Tyr	Val	Glu	Phe	Lys	
		260					265					270					
	Asp	Val	Gly	Trp	Gln	Asn	Trp	Val	Ile	Ala	Pro	Gln	Gly	Tyr	Met	Ala	
45		275				280					285					290	
	Asn	Tyr	Cys	Tyr	Gly	Glu	Cys	Pro	Tyr	Pro	Leu	Thr	Glu	Ile	Leu	Asn	
					295					300						305	

	Gly	Ser	Asn	His	Ala	Ile	Leu	Gln	Thr	Leu	Val	His	Ser	Ile	Glu	Pro
				310					315					320		
5	Glu	Asp	Ile	Pro	Leu	Pro	Cys	Cys	Val	Pro	Thr	Lys	Met	Ser	Pro	Ile
			325					330					335			
	Ser	Met	Leu	Phe	Tyr	Asp	Asn	Asn	Asp	Asn	Val	Val	Leu	Arg	His	Tyr
		340					345					350				
10	Glu	Asn	Met	Ala	Val	Asp	Glu	Cys	Gly	Cys	Arg					
	355			360			365									

15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 438 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: protein

25 (ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..438
(D) OTHER INFORMATION: /note= "PRE-PRO-VGR1"

30 (x) PUBLICATION INFORMATION:
(A) AUTHORS: LYONS,
(C) JOURNAL: Proc. Natl. Acad. Sci. U.S.A.
(D) VOLUME: 86
(F) PAGES: 4554-4558
(G) DATE: 1989

40	Met 1	Arg	Lys	Met	Gln 5	Lys	Glu	Ile	Leu	Ser 10	Val	Leu	Gly	Pro	Pro 15	His
	Arg	Pro	Arg	Pro 20	Leu	His	Gly	Leu	Gln 25	Gln	Pro	Gln	Pro	Pro 30	Val	Leu
45	Pro	Pro	Gln 35	Gln	Gln	Gln	Gln 40	Gln	Gln	Gln	Gln	Gln	Thr 45	Ala	Asp	Glu
	Glu 50	Pro	Pro	Pro	Gly	Arg 55	Leu	Lys	Ser	Ala	Pro	Leu	Phe 60	Met	Leu	Asp

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	Leu	Tyr	Asn	Ala	Leu	Ser	Asn	Asp	Asp	Glu	Glu	Asp	Gly	Ala	Ser	Glu
	65					70					75					80
5	Gly	Val	Gly	Gln	Glu	Pro	Gly	Ser	His	Gly	Gly	Ala	Ser	Ser	Ser	Gln
					85					90					95	
	Leu	Arg	Gln	Pro	Ser	Pro	Gly	Ala	Ala	His	Ser	Leu	Asn	Arg	Lys	Ser
				100					105					110		
10	Leu	Leu	Ala	Pro	Gly	Pro	Gly	Gly	Gly	Ala	Ser	Pro	Leu	Thr	Ser	Ala
			115					120						125		
	Gln	Asp	Ser	Ala	Phe	Leu	Asn	Asp	Ala	Asp	Met	Val	Met	Ser	Phe	Val
15		130					135					140				
	Asn	Leu	Val	Gly	Tyr	Asp	Lys	Glu	Phe	Ser	Pro	His	Gln	Arg	His	His
	145					150					155					160
20	Lys	Glu	Phe	Lys	Phe	Asn	Leu	Ser	Gln	Ile	Pro	Glu	Gly	Glu	Ala	Val
					165					170					175	
	Thr	Ala	Ala	Glu	Phe	Arg	Val	Tyr	Lys	Asp	Cys	Val	Val	Gly	Ser	Phe
				180					185					190		
25	Lys	Asn	Gln	Thr	Phe	Leu	Ile	Ser	Ile	Tyr	Gln	Val	Leu	Gln	Glu	Ala
			195					200					205			
	Gln	His	Arg	Asp	Ser	Asp	Leu	Phe	Leu	Leu	Asp	Thr	Arg	Val	Val	Trp
30		210					215					220				
	Ala	Ser	Glu	Glu	Gly	Trp	Leu	Glu	Phe	Asp	Ile	Thr	Ala	Thr	Ser	Asn
	225					230					235					240
35	Leu	Trp	Val	Val	Ile	Pro	Gln	His	Asn	Met	Gly	Leu	Gln	Leu	Ser	Val
					245					250					255	
	Val	Thr	Arg	Asp	Gly	Leu	His	Val	Asn	Pro	Arg	Ala	Ala	Gly	Leu	Val
				260					265					270		
40	Gly	Arg	Asp	Gly	Pro	Tyr	Asp	Lys	Gln	Pro	Phe	Met	Val	Ala	Phe	Phe
			275					280					285			
	Lys	Val	Ser	Glu	Val	His	Val	Arg	Thr	Thr	Arg	Ser	Ala	Ser	Ser	Arg
45		290					295					300				
	Arg	Arg	Gln	Gln	Ser	Arg	Asn	Arg	Ser	Thr	Gln	Ser	Gln	Asp	Val	Ser
	305					310					315					320
50	Arg	Gly	Ser	Gly	Ser	Ser	Asp	Tyr	Asn	Gly	Ser	Glu	Leu	Lys	Thr	Ala
					325					330					335	

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Cys Lys Lys His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Gln
 340 345 350
 5 Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly
 355 360 365
 Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
 370 375 380
 10 Ile Val Gln Thr Leu Val His Leu Met Asn Pro Glu Thr Val Pro Lys
 385 390 395 400
 Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe
 405 410 415
 15 Asp Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val
 420 425 430
 20 Arg Ala Cys Gly Cys His
 435

(2) INFORMATION FOR SEQ ID NO:15:

- 25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 372 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 30 (ii) MOLECULE TYPE: protein
 (ix) FEATURE:
 35 (A) NAME/KEY: Protein
 (B) LOCATION: 1..372
 (D) OTHER INFORMATION: /note= "PRE-PRO-GDF-1"
 (x) PUBLICATION INFORMATION:
 40 (A) AUTHORS: LEE,
 (B) TITLE: EXPRESSION OF GROWTH/DIFFERENTIATION FACTOR 1
 (C) JOURNAL: Proc. Natl. Acad. Sci. U.S.A.
 (D) VOLUME: 88
 (F) PAGES: 4250-4254
 (G) DATE: MAY-1991
 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

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	Met	Pro	Pro	Pro	Gln	Gln	Gly	Pro	Cys	Gly	His	His	Leu	Leu	Leu	Leu	
	1				5					10				15			
5	Leu	Ala	Leu	Leu	Leu	Pro	Ser	Leu	Pro	Leu	Thr	Arg	Ala	Pro	Val	Pro	
			20						25					30			
	Pro	Gly	Pro	Ala	Ala	Ala	Leu	Leu	Gln	Ala	Leu	Gly	Leu	Arg	Asp	Glu	
			35					40					45				
10	Pro	Gln	Gly	Ala	Pro	Arg	Leu	Arg	Pro	Val	Pro	Pro	Val	Met	Trp	Arg	
		50					55					60					
	Leu	Phe	Arg	Arg	Arg	Asp	Pro	Gln	Glu	Thr	Arg	Ser	Gly	Ser	Arg	Arg	
	65				70						75					80	
15	Thr	Ser	Pro	Gly	Val	Thr	Leu	Gln	Pro	Cys	His	Val	Glu	Glu	Leu	Gly	
					85					90					95		
	Val	Ala	Gly	Asn	Ile	Val	Arg	His	Ile	Pro	Asp	Arg	Gly	Ala	Pro	Thr	
20				100					105					110			
	Arg	Ala	Ser	Glu	Pro	Val	Ser	Ala	Ala	Gly	His	Cys	Pro	Glu	Trp	Thr	
			115					120					125				
25	Val	Val	Phe	Asp	Leu	Ser	Ala	Val	Glu	Pro	Ala	Glu	Arg	Pro	Ser	Arg	
		130					135					140					
	Ala	Arg	Leu	Glu	Leu	Arg	Phe	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Pro	Glu	
	145					150				155						160	
30	Gly	Gly	Trp	Glu	Leu	Ser	Val	Ala	Gln	Ala	Gly	Gln	Gly	Ala	Gly	Ala	
				165					170						175		
	Asp	Pro	Gly	Pro	Val	Leu	Leu	Arg	Gln	Leu	Val	Pro	Ala	Leu	Gly	Pro	
35				180					185					190			
	Pro	Val	Arg	Ala	Glu	Leu	Leu	Gly	Ala	Ala	Trp	Ala	Arg	Asn	Ala	Ser	
			195					200					205				
40	Trp	Pro	Arg	Ser	Leu	Arg	Leu	Ala	Leu	Ala	Leu	Arg	Pro	Arg	Ala	Pro	
		210					215					220					
	Ala	Ala	Cys	Ala	Arg	Leu	Ala	Glu	Ala	Ser	Leu	Leu	Leu	Val	Thr	Leu	
	225					230					235					240	
45	Asp	Pro	Arg	Leu	Cys	His	Pro	Leu	Ala	Arg	Pro	Arg	Arg	Asp	Ala	Glu	
				245						250				255			
	Pro	Val	Leu	Gly	Gly	Gly	Pro	Gly	Gly	Ala	Cys	Arg	Ala	Arg	Arg	Leu	
50				260					265					270			

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Tyr Val Ser Phe Arg Glu Val Gly Trp His Arg Trp Val Ile Ala Pro
 275 280 285
 5 Arg Gly Phe Leu Ala Asn Tyr Cys Gln Gly Gln Cys Ala Leu Pro Val
 290 295 300
 Ala Leu Ser Gly Ser Gly Gly Pro Pro Ala Leu Asn His Ala Val Leu
 305 310 315 320
 10 Arg Ala Leu Met His Ala Ala Ala Pro Gly Ala Ala Asp Leu Pro Cys
 325 330 335
 Cys Val Pro Ala Arg Leu Ser Pro Ile Ser Val Leu Phe Phe Asp Asn
 340 345 350
 15 Ser Asp Asn Val Val Leu Arg Gln Tyr Glu Asp Met Val Val Asp Glu
 355 360 365
 20 Cys Gly Cys Arg
 370

(2) INFORMATION FOR SEQ ID NO:16:

- 25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 455 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 30 (ii) MOLECULE TYPE: protein
 (ix) FEATURE:
 35 (A) NAME/KEY: Protein
 (B) LOCATION: 1..455
 (D) OTHER INFORMATION: /note= "PRE-PRO 60A"
 (x) PUBLICATION INFORMATION:
 40 (A) AUTHORS: WHARTON,
 (C) JOURNAL: Proc. Natl. Acad. Sci. U.S.A.
 (D) VOLUME: 88
 (F) PAGES: 9214-9218
 (G) DATE: 1991
 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met Ser Gly Leu Arg Asn Thr Ser Glu Ala Val Ala Val Leu Ala Ser
 1 5 10 15

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	Leu	Gly	Leu	Gly	Met	Val	Leu	Leu	Met	Phe	Val	Ala	Thr	Thr	Pro	Pro
			20						25					30		
5	Ala	Val	Glu	Ala	Thr	Gln	Ser	Gly	Ile	Tyr	Ile	Asp	Asn	Gly	Lys	Asp
			35					40				45				
	Gln	Thr	Ile	Met	His	Arg	Val	Leu	Ser	Glu	Asp	Asp	Lys	Leu	Asp	Val
		50					55					60				
10	Ser	Tyr	Glu	Ile	Leu	Glu	Phe	Leu	Gly	Ile	Ala	Glu	Arg	Pro	Thr	His
	65					70					75					80
	Leu	Ser	Ser	His	Gln	Leu	Ser	Leu	Arg	Lys	Ser	Ala	Pro	Lys	Phe	Leu
15					85					90					95	
	Leu	Asp	Val	Tyr	His	Arg	Ile	Thr	Ala	Glu	Glu	Gly	Leu	Ser	Asp	Gln
				100					105					110		
20	Asp	Glu	Asp	Asp	Asp	Tyr	Glu	Arg	Gly	His	Arg	Ser	Arg	Arg	Ser	Ala
			115					120					125			
	Asp	Leu	Glu	Glu	Asp	Glu	Gly	Glu	Gln	Gln	Lys	Asn	Phe	Ile	Thr	Asp
		130					135					140				
25	Leu	Asp	Lys	Arg	Ala	Ile	Asp	Glu	Ser	Asp	Ile	Ile	Met	Thr	Phe	Leu
	145					150					155					160
	Asn	Lys	Arg	His	His	Asn	Val	Asp	Glu	Leu	Arg	His	Glu	His	Gly	Arg
30					165					170					175	
	Arg	Leu	Trp	Phe	Asp	Val	Ser	Asn	Val	Pro	Asn	Asp	Asn	Tyr	Leu	Val
				180					185					190		
35	Met	Ala	Glu	Leu	Arg	Ile	Tyr	Gln	Asn	Ala	Asn	Glu	Gly	Lys	Trp	Leu
			195					200					205			
	Thr	Ala	Asn	Arg	Glu	Phe	Thr	Ile	Thr	Val	Tyr	Ala	Ile	Gly	Thr	Gly
		210					215					220				
40	Thr	Leu	Gly	Gln	His	Thr	Met	Glu	Pro	Leu	Ser	Ser	Val	Asn	Thr	Thr
	225					230					235					240
	Gly	Asp	Tyr	Val	Gly	Trp	Leu	Glu	Leu	Asn	Val	Thr	Glu	Gly	Leu	His
45					245					250					255	
	Glu	Trp	Leu	Val	Lys	Ser	Lys	Asp	Asn	His	Gly	Ile	Tyr	Ile	Gly	Ala
				260					265					270		
50	His	Ala	Val	Asn	Arg	Pro	Asp	Arg	Glu	Val	Lys	Leu	Asp	Asp	Ile	Gly
			275					280					285			

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Leu Ile His Arg Lys Val Asp Asp Glu Phe Gln Pro Phe Met Ile Gly
 290 295 300
 5 Phe Phe Arg Gly Pro Glu Leu Ile Lys Ala Thr Ala His Ser Ser His
 305 310 315 320
 His Arg Ser Lys Arg Ser Ala Ser His Pro Arg Lys Arg Lys Lys Ser
 325 330 335
 10 Val Ser Pro Asn Asn Val Pro Leu Leu Glu Pro Met Glu Ser Thr Arg
 340 345 350
 Ser Cys Gln Met Gln Thr Leu Tyr Ile Asp Phe Lys Asp Leu Gly Trp
 355 360 365
 15 His Asp Trp Ile Ile Ala Pro Glu Gly Tyr Gly Ala Phe Tyr Cys Ser
 370 375 380
 Gly Glu Cys Asn Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His
 385 390 395 400
 20 Ala Ile Val Gln Thr Leu Val His Leu Leu Glu Pro Lys-Lys Val Pro
 405 410 415
 Lys Pro Cys Cys Ala Pro Thr Arg Leu Gly Ala Leu Pro Val Leu Tyr
 420 425 430
 25 His Leu Asn Asp Glu Asn Val Asn Leu Lys Lys Tyr Arg Asn Met Ile
 435 440 445
 30 Val Lys Ser Cys Gly Cys His
 450 455

(2) INFORMATION FOR SEQ ID NO:17:

35

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 472 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE: protein

45

(ix) FEATURE:

- (A) NAME/KEY: Protein
 (B) LOCATION: 1..472
 (D) OTHER INFORMATION: /note= "PRE-PRO-BMP3"

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(x) PUBLICATION INFORMATION:

5 (A) AUTHORS: WOZNEY,
 (C) JOURNAL: SCIENCE
 (D) VOLUME: 242
 (F) PAGES: 1528-1534
 (G) DATE: 1988

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

10 Met Ala Gly Ala Ser Arg Leu Leu Phe Leu Trp Leu Gly Cys Phe Cys
 1 5 10 15
 Val Ser Leu Ala Gln Gly Glu Arg Pro Lys Pro Pro Phe Pro Glu Leu
 20 25 30
 15 Arg Lys Ala Val Pro Gly Asp Arg Thr Ala Gly Gly Gly Pro Asp Ser
 35 40 45
 Glu Leu Gln Pro Gln Asp Lys Val Ser Glu His Met Leu Arg Leu Tyr
 20 50 55 60
 Asp Arg Tyr Ser Thr Val Gln Ala Ala Arg Thr Pro Gly Ser Leu Glu
 65 70 75 80
 25 Gly Gly Ser Gln Pro Trp Arg Pro Arg Leu Leu Arg Glu Gly Asn Thr
 85 90 95
 Val Arg Ser Phe Arg Ala Ala Ala Ala Glu Thr Leu Glu Arg Lys Gly Leu
 100 105 110
 30 Tyr Ile Phe Asn Leu Thr Ser Leu Thr Lys Ser Glu Asn Ile Leu Ser
 115 120 125
 Ala Thr Leu Tyr Phe Cys Ile Gly Glu Leu Gly Asn Ile Ser Leu Ser
 130 135 140
 35 Cys Pro Val Ser Gly Gly Cys Ser His His Ala Gln Arg Lys His Ile
 145 150 155
 40 Gln Ile Asp Leu Ser Ala Trp Thr Leu Lys Phe Ser Arg Asn Gln Ser
 160 165 170 175
 Gln Leu Leu Gly His Leu Ser Val Asp Met Ala Lys Ser His Arg Asp
 180 185 190
 45 Ile Met Ser Trp Leu Ser Lys Asp Ile Thr Gln Phe Leu Arg Lys Ala
 195 200 205
 50 Lys Glu Asn Glu Glu Phe Leu Ile Gly Phe Asn Ile Thr Ser Lys Gly
 210 215 220

[illegible]

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(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 453 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

10

(ix) FEATURE:

- (A) NAME/KEY: Protein
 (B) LOCATION: 1..453
 15 (D) OTHER INFORMATION: /note= "PRE-PRO-BMP5 (HUMAN)"

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: CELESTE,
 (C) JOURNAL: Proc. Natl. Acad. Sci. U.S.A.
 20 (D) VOLUME: 87
 (F) PAGES: 9843-9847
 (G) DATE: 1991

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

25

Met His Leu Thr Val Phe Leu Leu Lys Gly Ile Val Gly Phe Leu Trp
 1 5 10 15

30

Ser Cys Trp Val Leu Val Gly Tyr Ala Lys Gly Gly Leu Gly Asp Asn
 20 25 30

His Val His Ser Ser Phe Ile Tyr Arg Arg Leu Arg Asn His Glu Arg
 35 40 45

35

Arg Glu Ile Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg
 50 55 60

40

Pro Arg Pro Phe Ser Pro Gly Lys Gln Ala Ser Ser Ala Pro Leu Phe
 65 70 75 80

Met Leu Asp Leu Tyr Asn Ala Met Thr Asn Glu Glu Asn Pro Glu Glu
 85 90 95

45

Ser Glu Tyr Ser Val Arg Ala Ser Leu Ala Glu Glu Thr Arg Gly Ala
 100 105 110

Arg Lys Gly Tyr Pro Ala Ser Pro Asn Gly Tyr Pro Arg Arg Ile
 115 120 125

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	Gln	Leu	Ser	Arg	Thr	Thr	Pro	Leu	Thr	Thr	Gln	Ser	Pro	Pro	Leu	Ala	
			130					135					140				
5	Ser	Leu	His	Asp	Thr	Asn	Phe	Leu	Asn	Asp	Ala	Asp	Met	Val	Met	Ser	
		145					150					155					
	Phe	Val	Asn	Leu	Val	Glu	Arg	Asp	Lys	Asp	Phe	Ser	His	Gln	Arg	Arg	
	160					165					170					175	
10	His	Tyr	Lys	Glu	Arg	Phe	Asp	Leu	Thr	Gln	Ile	Pro	His	Gly	Glu	Ala	Val
					180					185					190		
	Thr	Ala	Ala	Glu	Phe	Arg	Ile	Val	Lys	Asp	Arg	Ser	Asn	Asn	Arg	Phe	
15			195					200					205				
	Glu	Asn	Glu	Thr	Ile	Lys	Ile	Ser	Ile	Tyr	Gln	Ile	Ile	Lys	Glu	Tyr	
		210					215					220					
	Thr	Asn	Arg	Asp	Ala	Asp	Leu	Phe	Leu	Leu	Asp	Thr	Arg	Lys	Ala	Gln	
20	225					230					235					240	
	Ala	Leu	Asp	Val	Gly	Trp	Leu	Val	Phe	Asp	Ile	Thr	Val	Thr	Ser	Asn	
					245					250					255		
25	His	Trp	Val	Ile	Asn	Pro	Gln	Asn	Asn	Leu	Gly	Leu	Gln	Leu	Cys	Ala	
				260					265					270			
	Glu	Thr	Gly	Asp	Gly	Arg	Ser	Ile	Asn	Val	Lys	Ser	Ala	Gly	Leu	Val	
30			275					280					285				
	Gly	Arg	Gln	Gly	Pro	Gln	Ser	Lys	Gln	Pro	Phe	Met	Val	Ala	Phe	Phe	
		290					295					300					
	Lys	Ala	Ser	Glu	Val	Leu	Leu	Arg	Ser	Val	Arg	Ala	Ala	Asn	Lys	Arg	
35	305					310					315					320	
	Lys	Asn	Gln	Asn	Arg	Asn	Lys	Ser	Ser	Ser	His	Gln	Asp	Ser	Ser	Arg	
					325					330					335		
40	Met	Ser	Ser	Val	Gly	Asp	Tyr	Asn	Thr	Ser	Glu	Gln	Lys	Gln	Ala	Cys	
				340					345					350			
	Lys	Lys	His	Glu	Leu	Tyr	Val	Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln	Asp	
45			355					360					365				
	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala	Ala	Phe	Tyr	Cys	Asp	Gly	Glu	
		370					375					380					
50	Cys	Ser	Phe	Pro	Leu	Asn	Ala	His	Met	Asn	Ala	Thr	Asn	His	Ala	Ile	
	385					390					395					400	

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[illegible]

(2) INFORMATION FOR SEQ ID NO:19:

15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 513 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: protein

25 (ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..513
(D) OTHER INFORMATION: /note= "PRE-PRO-BMP6 (HUMAN)"

30 (x) PUBLICATION INFORMATION:
(A) AUTHORS: CELESTE,
(C) JOURNAL: Proc. Natl. Acad. Sci. U.S.A.
(D) VOLUME: 87
(F) PAGES: 9843-9847
(G) DATE: 1991

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met Pro Gly Leu Gly Arg Arg Ala Gln Trp Leu Cys Trp Trp Trp Gly
 1           5           10           15

40 Leu Leu Cys Ser Cys Cys Gly Pro Pro Pro Leu Arg Pro Pro Leu Pro
    20           25           30

45 Ala Ala Ala Ala Ala Ala Ala Gly Gly Gln Leu Leu Gly Asp Gly Gly
    35           40           45

Ser Pro Gly Arg Thr Glu Gln Pro Pro Pro Ser Pro Gln Ser Ser Ser
 50           55           60

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	Gly	Phe	Leu	Tyr	Arg	Arg	Leu	Lys	Thr	Gln	Glu	Lys	Arg	Glu	Met	Gln	
	65					70					75					80	
5	Lys	Glu	Ile	Leu	Ser	Val	Leu	Gly	Leu	Pro	His	Arg	Pro	Arg	Pro	Leu	
					85					90					95		
	His	Gly	Leu	Gln	Gln	Pro	Gln	Pro	Pro	Ala	Leu	Arg	Gln	Gln	Glu	Glu	
				100					105					110			
10	Gln	Gln	Gln	Gln	Gln	Gln	Leu	Pro	Arg	Gly	Glu	Pro	Pro	Pro	Gly	Arg	
				115				120					125				
	Leu	Lys	Ser	Ala	Pro	Leu	Phe	Met	Leu	Asp	Leu	Tyr	Asn	Ala	Leu	Ser	
15		130					135					140					
	Ala	Asp	Asn	Asp	Glu	Asp	Gly	Ala	Ser	Glu	Gly	Glu	Arg	Gln	Gln	Ser	
	145					150					155					160	
20	Trp	Pro	His	Glu	Ala	Ala	Ser	Ser	Ser	Gln	Arg	Arg	Gln	Pro	Pro	Pro	
					165					170					175		
	Gly	Ala	Ala	His	Pro	Leu	Asn	Arg	Lys	Ser	Leu	Leu	Ala	Pro	Gly	Ser	
				180					185					190			
25	Gly	Ser	Gly	Gly	Ala	Ser	Pro	Leu	Thr	Ser	Ala	Gln	Asp	Ser	Ala	Phe	
			195					200					205				
	Leu	Asn	Asp	Ala	Asp	Met	Val	Met	Ser	Phe	Val	Asn	Leu	Val	Glu	Tyr	
30		210					215					220					
	Asp	Lys	Glu	Phe	Ser	Pro	Arg	Gln	Arg	His	His	Lys	Glu	Phe	Lys	Phe	
	225					230					235					240	
35	Asn	Leu	Ser	Gln	Ile	Pro	Glu	Gly	Glu	Val	Val	Thr	Ala	Ala	Glu	Phe	
					245					250					255		
	Arg	Ile	Val	Lys	Asp	Cys	Val	Met	Gly	Ser	Phe	Lys	Asn	Gln	Thr	Phe	
				260					265					270			
40	Leu	Ile	Ser	Ile	Tyr	Gln	Val	Leu	Gln	Glu	His	Gln	His	Arg	Asp	Ser	
			275					280					285				
	Asp	Leu	Phe	Leu	Leu	Asp	Thr	Arg	Val	Val	Trp	Ala	Ser	Glu	Glu	Gly	
45		290					295					300					
	Trp	Leu	Glu	Phe	Asp	Ile	Thr	Ala	Thr	Ser	Asn	Leu	Trp	Val	Val	Thr	
	305					310					315					320	

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	Pro	Gln	His	Asn	Met	Gly	Leu	Gln	Leu	Ser	Val	Val	Thr	Arg	Asp	Gly	
					325											335	
5	Val	His	Val	His	Pro	Arg	Ala	Ala	Gly	Leu	Val	Gly	Arg	Asp	Gly	Pro	
				340					345					350			
	Tyr	Asp	Lys	Gln	Pro	Phe	Met	Val	Ala	Phe	Phe	Lys	Val	Ser	Glu		
			355					360					365				
10	Val	His	Val	Arg	Thr	Thr	Arg	Ser	Ala	Ser	Ser	Arg	Arg	Arg	Gln	Gln	
			370					375					380				
	Ser	Arg	Asn	Arg	Ser	Thr	Gln	Ser	Gln	Asp	Val	Ala	Arg	Val	Ser	Ser	
15			385				390					395					
	Ala	Ser	Asp	Tyr	Asn	Ser	Ser	Glu	Leu	Lys	Thr	Ala	Cys	Arg	Lys	His	
	400					405					410					415	
20	Glu	Leu	Tyr	Val	Ser	Phe	Gln	Asp	Leu	Gly	Trp	Gln	Asp	Trp	Ile	Ile	
				420						425					430		
	Ala	Pro	Lys	Gly	Tyr	Ala	Ala	Asn	Tyr	Cys	Asp	Gly	Glu	Cys	Ser	Phe	
				435					440					445			
25	Pro	Leu	Asn	Ala	His	Met	Asn	Ala	Thr	Asn	His	Ala	Ile	Val	Gln	Thr	
			450					455					460				
	Leu	Val	His	Leu	Met	Asn	Pro	Glu	Tyr	Val	Pro	Lys	Pro	Cys	Cys	Ala	Pro
30		465					470					475					480
	Thr	Lys	Leu	Asn	Ala	Ile	Ser	Val	Leu	Tyr	Phe	Asp	Asp	Asn	Ser	Asn	
					485					490					495		
35	Val	Ile	Leu	Lys	Lys	Tyr	Arg	Asn	Met	Val	Val	Arg	Ala	Cys	Gly	Cys	
				500					505					510			

His

40 (2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 97 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

50

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(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..97

(D) OTHER INFORMATION: /label= Generic-Seq-7

5 /note= "wherein each Xaa is independently selected
from a group of one or more specified amino acids
as defined in the specification."

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Leu Xaa Xaa Xaa Phe Xaa Xaa Xaa Gly Trp Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15

15 Pro Xaa Xaa Xaa Xaa Ala Xaa Tyr Cys Xaa Gly Xaa Cys Xaa Xaa Pro
 20 25 30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn His Ala Xaa Xaa Xaa Xaa Xaa
35 40 45

20 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Cys Xaa Pro
50 55 60

25 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa
65 70 75 80

Val Xaa Leu Xaa Xaa Xaa Xaa Met Xaa Val Xaa Xaa Cys Xaa Cys
85 90 95

30 Xaa

(2) INFORMATION FOR SEQ ID NO:21:

35 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: protein

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(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..102

(D) OTHER INFORMATION: /label= Generic-Seq-8

5 /note= "wherein each Xaa is independently selected
from a group of one or more specified amino acids
as defined in the specification."

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Cys Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Phe Xaa Xaa Xaa Gly Trp Xaa
1 5 10 15
Xaa Xaa Xaa Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala Xaa Tyr Cys Xaa Gly
20 25 30
Xaa Cys Xaa Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn His Ala
35 40 45
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
50 55 60
Xaa Cys Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa
65 70 75 80
Xaa Xaa Xaa Xaa Xaa Val Xaa Leu Xaa Xaa Xaa Xaa Xaa Met Xaa Val
85 90 95
Xaa Xaa Cys Xaa Cys Xaa
100

30

(2) INFORMATION FOR SEQ ID NO:22:

35

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

45

(A) NAME/KEY: Protein

(B) LOCATION: 1..102

(D) OTHER INFORMATION: /label= OPX

50 /note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED
FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS
AS DEFINED IN THE SPECIFICATION (SECTION II.B.2.)"

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

5 Cys Xaa Xaa His Glu Leu Tyr Val Xaa Phe Xaa Asp Leu Gly Trp Xaa
 1 5 10 15
 Asp Trp Xaa Ile Ala Pro Xaa Gly Tyr Xaa Ala Tyr Tyr Cys Glu Gly
 20 25 30
 10 Glu Cys Xaa Phe Pro Leu Xaa Ser Xaa Met Asn Ala Thr Asn His Ala
 35 40 45
 Ile Xaa Gln Xaa Leu Val His Xaa Xaa Xaa Pro Xaa Xaa Val Pro Lys
 50 55 60
 15 Xaa Cys Cys Ala Pro Thr Xaa Leu Xaa Ala Xaa Ser Val Leu Tyr Xaa
 65 70 75 80
 Asp Xaa Ser Xaa Asn Val Xaa Leu Xaa Lys Xaa Arg Asn Met Val Val
 85 90 95
 20 Xaa Ala Cys Gly Cys His
 100

(2) INFORMATION FOR SEQ ID NO:23:

25

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 30 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

35

(ix) FEATURE:

- (A) NAME/KEY: Cleavage-site
 (B) LOCATION: 1..4
 (D) OTHER INFORMATION: /note= "PROTEOLYTIC CLEAVAGE SITE"

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Arg Xaa Xaa Arg
 1

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What is claimed is:

1. Dimeric protein comprising a pair of protein subunits associated to defined a dimeric structure having morphogenic activity,
each of said subunits comprising at least a 100 amino acid sequence having a pattern of cysteine residues characteristic of the morphogen family,
at least one of said subunits comprising a mature form of a subunit of a member of the morphogen family, or an allelic, species, or sequence variant thereof, noncovalently complexed with
a peptide comprising a pro region of a member of the morphogen family, or an allelic, species, or sequence variant thereof to form a complex which is more soluble in aqueous solvents than the uncomplexed pair of subunits.
2. The protein of claim 1 wherein both said subunits comprise a mature form of a subunit of a member of the morphogen family or an allelic, species, or sequence variant thereof, each said subunit being noncovalently complexed with a said peptide.
3. The protein of claim 1 wherein each said subunit is the mature form of human OP-1, or a species or allelic variant thereof.
4. The protein of claim 1, 2, or 3 wherein the peptide comprises the pro region of human OP-1, or a species, allelic or sequence variant thereof.

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5. The protein of claim 1 wherein said peptide comprises at least the first 18 amino acids of an amino acid sequence defining said pro region.

6. The protein of claim 1 wherein said peptide comprises at least the first 18 amino acids of an amino acid sequence defining said pro region in Seq. ID Nos. 1-16 or a sequence variant thereof.

7. The protein of claim 1 or 6 wherein said peptide comprises the full length form of said pro region.

8. The protein of claim 1 wherein said pro region peptide comprises an amino acid sequence selected from sequences defined by residues 30-48, 30-292 and 48-292 of Seq. ID No. 1.

9. The protein of claim 1 wherein said pro region peptide comprises an amino acid sequence encoded by a nucleic acid that hybridizes under stringent conditions with a DNA encoding the N-terminal 18 amino acids of the pro region sequences for Seq. ID Nos. 1-19.

10. The protein of claims 1 or 9 wherein said pro region peptide comprises a nucleic acid that hybridizes under stringent conditions with a DNA defined by nucleotides of 136-192 of Seq. ID No. 1 or nucleotides 157-211 of Seq. ID No. 5.

11. The protein of claim 1 wherein said subunit sequence variant comprises a chimeric morphogen amino acid sequence.

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12. The protein of claim 1 wherein said peptide comprises a chimeric pro region amino acid sequence.
13. The protein of claim 1 wherein said subunit comprises a sequence defined by Generic Sequence 7 or Generic Sequence 8.
14. The protein of claim 1 wherein said subunit comprises a sequence having 60% amino acid identity with the sequence defined by residues 335-431 of Seq. ID No.1.
15. The protein of claim 1 wherein said subunit comprises the mature form of a subunit defined by any of the sequences of Seq. ID No. 5-19.
16. The protein of claim 1 wherein said subunit comprises an amino acid sequence encoded by a nucleic acid that hybridizes with a DNA defined by nucleotides 1036-1341 of Seq. ID No. 1 or nucleotides 1390-1695 of Seq. ID No. 5.
17. The protein of claim 1 further comprising an molecule capable of enhancing the stability of said complex.
18. A therapeutic composition comprising the protein of any of claims 1, 2, 5-9 or 11-17.
19. A therapeutic composition comprising the protein of claim 1 wherein each said subunit is the mature form of human OP-1, or a species or allelic variant thereof.

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20. A therapeutic composition comprising the protein of claim 1, wherein said peptide comprises part or all of the pro region of human OP-1, or a species or allelic variant thereof.

21. The therapeutic composition of claim 18 comprising the protein of claim 1 wherein said subunit comprises the mature form of a subunit defined by any of the sequences of Seq. ID Nos. 5-19.

22. A therapeutic composition comprising the protein of claims 3, 4 or 10.

23. The therapeutic composition of claims 18 or 22 further comprising a cofactor.

24. The therapeutic composition of claim 23 wherein said cofactor is a symptom-alleviating cofactor.

25. A kit for diagnosing a tissue disorder or evaluating the efficacy of a therapy to regenerate lost or damaged tissue in a mammal, the kit comprising:

- a) means for capturing a cell or fluid sample from said mammal,
- b) a binding protein capable of interacting specifically with a soluble morphogen complex in said sample, and
- c) means for detecting the binding protein bound to said soluble morphogen complex.

26. The kit of claim 25 wherein said binding protein is an antibody.

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27. A method for evaluating the status of a tissue, the method comprising the step of comparing the quantity of morphogen in a body fluid sample with the quantity of morphogen in a control sample.

28. A method for evaluating the efficacy of a therapy to regenerate lost or damaged tissue in a mammal, the method comprising the step of comparing the quantity of morphogen in a body fluid sample with the quantity of morphogen in a control sample.

29. A method for diagnosing a tissue disorder in a mammal, the method comprising the step of comparing the quantity of morphogen in a body fluid sample with the quantity of morphogen in a control sample.

30. The invention of claim 25, 26, 27 or 28 wherein said morphogen is a dimeric protein comprising a pair of protein subunits associated to defined a dimeric structure having morphogenic activity,

each of said subunits comprising at least a 100 amino acid sequence having a pattern of cysteine residues characteristic of the morphogen family,

at least one of said subunits comprising a mature form of a subunit of a member of the morphogen family, or an allelic, species, or sequence variant thereof, noncovalently complexed with

a peptide comprising a pro region of a member of the morphogen family, or an allelic, species, or sequence variant thereof to form a complex which is more soluble in aqueous solvents than the uncomplexed pair of subunits.

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31. The invention of claims 25, 26, 27 or 28 wherein said quantity of morphogen is detected by an immunoassay.

32. The invention of claims 25, 26, 27 or 28 wherein said quantity of morphogen is detected by an antibody capable of distinguishing soluble morphogen in a sample fluid.

33. The invention of claims 25, 26, 27 or 28 wherein said body fluid sample comprises serum.

34. The invention of claims 25 or 28 wherein said tissue disorder is a bone tissue disorder.

35. The invention of claim 34 wherein said bone tissue disorder is selected from the group consisting of osteosarcoma, osteoporosis, and Paget's disease.

36. A method of evaluating the status of a tissue, the method comprising the step of detecting the presence of anti-morphogen antibody in a tissue or body fluid sample.

37. A method for evaluating the efficacy of a therapy to regenerate lost or damaged tissue, the method comprising the step of detecting the presence of anti-morphogen antibody in a tissue or body fluid sample.

38. A method for diagnosing a tissue disorder, the method comprising the step of detecting the presence of anti-morphogen antibody in a tissue or body fluid sample.

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39. A kit for diagnosing a tissue disorder or evaluating the efficacy of a therapy to regenerate lost or damaged tissue in a mammal, the kit comprising:

- a) means for capturing a cell or fluid sample from said mammal;
- b) a binding protein capable of interacting specifically with an endogenous anti-morphogen antibody in said sample; and
- c) means for detecting said binding protein-bound to said endogenous anti-morphogen antibody.

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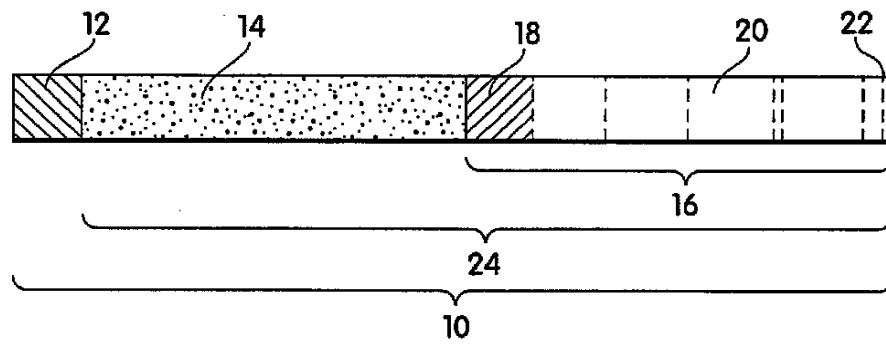


Fig. 1

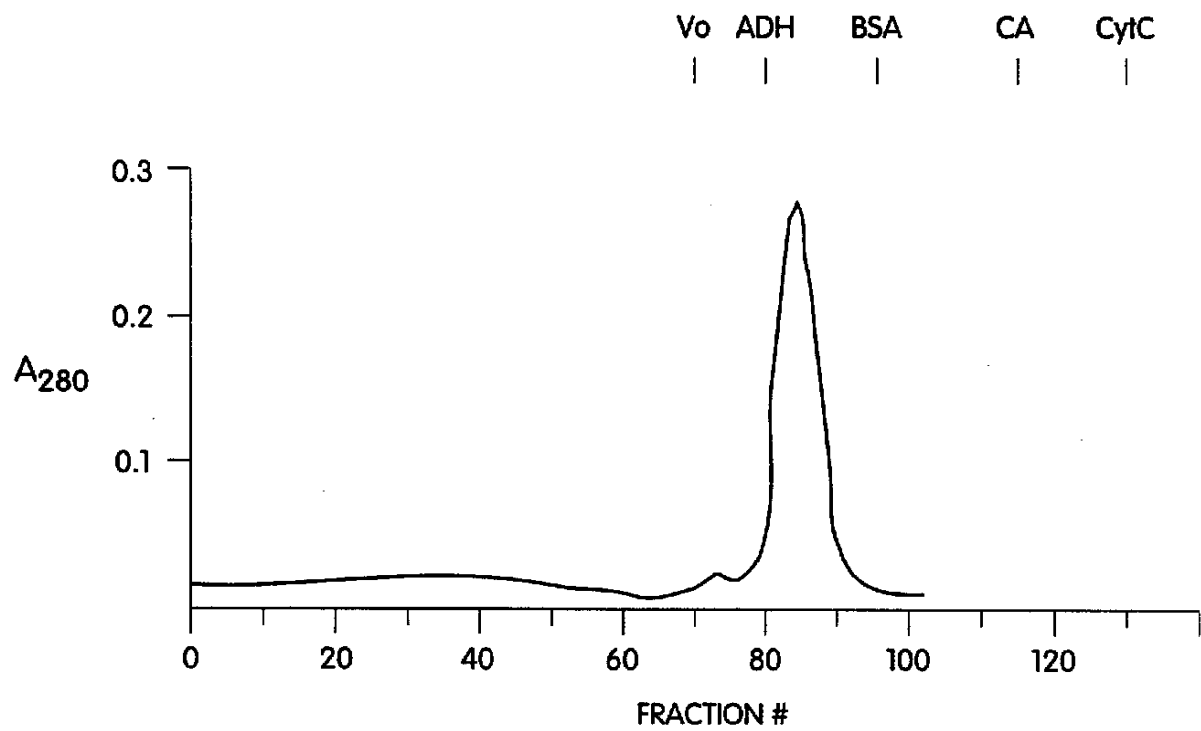


Fig. 3

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OP-2: RAPRSQQPFVVTFFRASPSPIRTTPRAVRPLRRRQPKKSNELPQANRLPGIFDDVHGSHGRQVC
 OP-1: RSIRSTGSKQRSQNRSKTPKNQEALRMANVAENSSSDQRQAC
 Vgr-1: RTTRSASSRRRQQSRNRSTQSDVSRGSGSSDYNGSELKTAC
 BMP-5: RSVRAANKRKNQNRNKSSSHQDSSRMSSVGDYNTSEQKQAC
 60A: RSKRSASHPRKRKKSvspnnvpllepmestrsc
 DPP: RSIRDVSGEGGKGGRNKRHRARRPTRRKNHDDTC
 BMP-2: RHVRISRSLHQDEHSWSQIRPLLVTFGHDGKGHPLHK--REKRQAKH--KQRRLKSSC
 BMP-4: RISRSLPQSGNWAQLRPLLVTFGHDGRGHALTRRRRAKRSPKHHSQARAKKNKNC
 Vg-1: RCKRPRRRKRSYSKLPFTASNIC
 BMP-3: RKKRSTGVLLPLQ.....KSKNKKKQKRGPHRKSQTLQFDEQTLKKARRKQWIEPRNC

Fig. 2

INTERNATIONAL SEARCH REPORT

Int. l. Application No
PCT/US 93/07189

A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 C12N15/12 A61K37/02 G01N33/50 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C07K C12N A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,91 18047 (GENENTECH, INC.) 28 November 1991 see page 2, line 24 - page 3, line 4 see page 4, line 4 - line 8 see page 5, line 16 - page 6, line 5 ---	1,5,7,12
X	MOLECULAR ENDOCRINOLOGY vol. 5, no. 1, January 1991 pages 149 - 155 R. GLENN HAMMONDS, JR. ET AL. 'Bone-inducing activity of mature BMP-2b produced from a hybrid BMP-2a/2b precursor' see abstract see page 149, right column, paragraph 3 - page 150, left column, paragraph 3 see page 152, left column, paragraph 2 - right column, paragraph 3 --- -/--	1,5,7,12

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

2 November 1993

Date of mailing of the international search report

14. 12. 93

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MONTERO LOPEZ, B

INTERNATIONAL SEARCH REPORT

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PCT/US 93/07189

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	WO,A,92 07073 (CREATIVE BIOMOLECULES, INC.) 30 April 1992 see page 6, line 2 - line 8 see page 7, line 4 - page 9, line 9 ---	1-16
P,X	WO,A,93 05751 (CREATIVE BIOMOLECULES, INC.) 1 April 1993 see page 7, line 16 - line 33 see page 10, line 5 - line 28 see page 11, line 6 - page 32, line 3 see page 37, line 17 - line 35 -----	1-24

INTERNATIONAL SEARCH REPORT

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International Application No

PCT/US 93/07189

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WO-A-9305751	01-04-93	AU-A- 2564592 AU-A- 3176293 WO-A- 9304692 AU-A- 2862492 WO-A- 9305172	05-04-93 27-04-93 18-03-93 05-04-93 18-03-93